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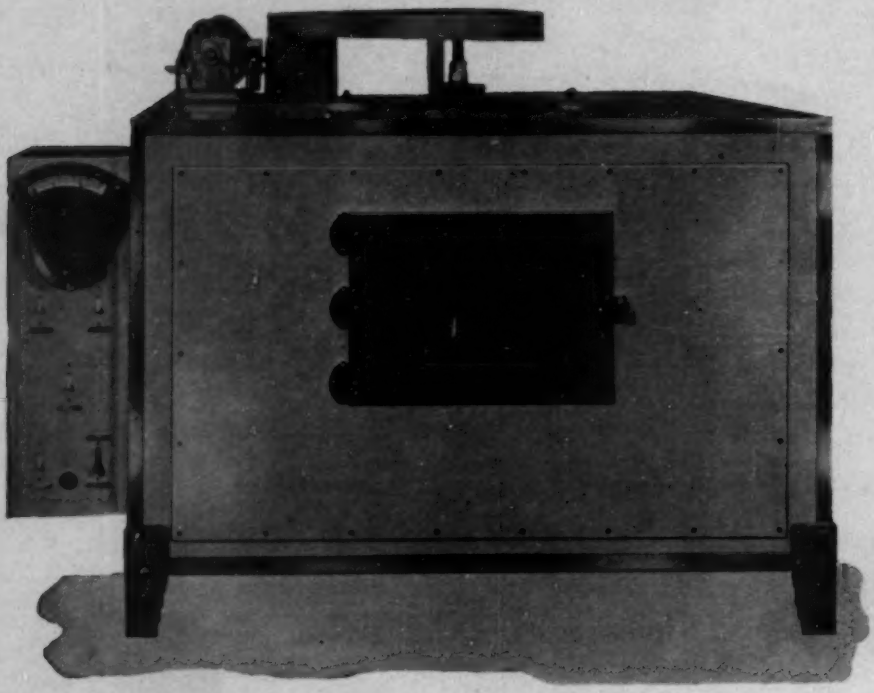

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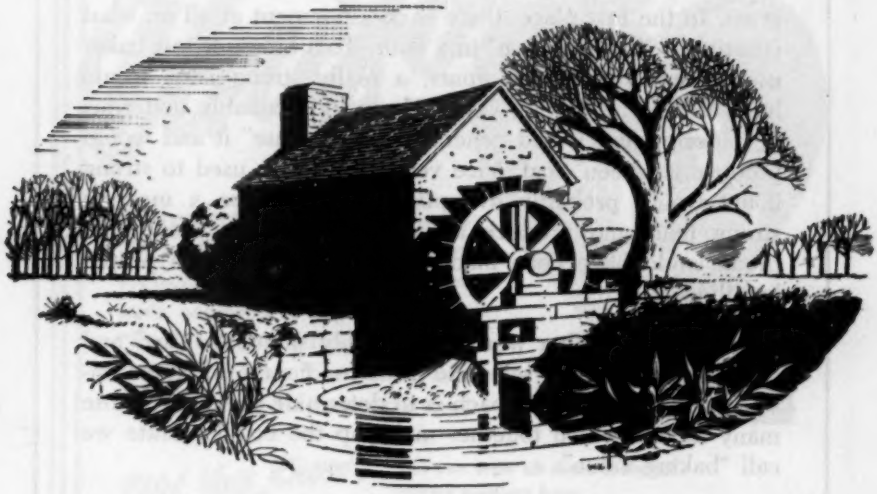

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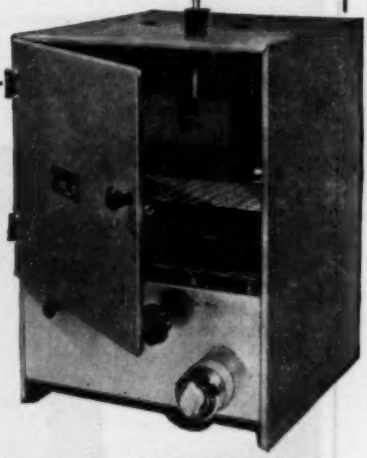

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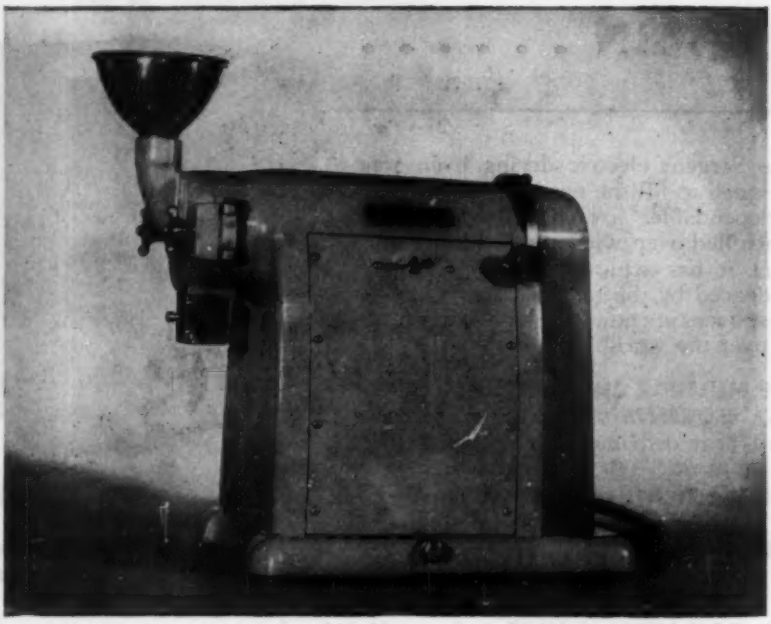

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CEREAL CHEMISTRY

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MICRO-DETERMINATION OF ENDOSPERM DENSITY AS A MEANS OF MAPPING MOISTURE DISTRIBUTION IN WHEAT GRAINS[1,2]

C. R. JONES AND J. D. CAMPBELL

ABSTRACT

The densities of small particles, dissected under oil from specified points within the endosperm, are determined to within 0.001 by suspension in water-saturated mixtures of carbon tetrachloride and cyclohexane. Over the useful range the density is linearly related to moisture content, the gradient being 0.0041 per 1% difference in moisture content. At 12% moisture content, vitreous grains of many different wheats showed similar values, close to 1.460 (25°/25° in air), for density of central endosperm.

A first application of the method indicated that at 20°C., moisture movement into the cheek centers of damped Manitoba grains was 85% complete in 24 hours.

The aim of this work was to develop a method for finding the level of moisture content at any specified point within the endosperm of a wheat grain. Previous workers, notably Ugrimoff, have followed penetration of water into immersed wheat by means of colorimetric observations on sectioned grains (6). Their conclusions, however, apply only to soaked grain and do not bear directly on milling practice, where wheat is conditioned by moistening to the extent of only about 5%, the added moisture being allowed to penetrate the interior of individual grains during a period of storage. This period depends upon temperature but practice varies widely and there is a scarcity of fundamental information. Recent developments in conditioning processes particularly call for methods of ascertaining moisture distribution; it is realized increasingly that the milling behavior of wheat at a specified moisture content may vary greatly according to the manner in which the moisture is held. Investigations have been made by following changes in physical behavior of grain, e.g., its electrical response and the rate of evaporation from it (7). Alterna-

[1] Manuscript received December 16, 1952.
[2] Contribution from the Research Association of British Flour Millers, St. Albans, England.

tively the moisture contents of "bran" and endosperm have been compared by making oven determinations on fractions rapidly separated from wheat by means of grinding and sifting (4, 12). Such methods indicate only the partition of moisture between outer and inner zones of the grain in a broad sense, i.e., they cannot discriminate between particular regions of the endosperm, and they may be subject to uncertainties, e.g., the apportionment of evaporative loss during the grinding and sifting procedure.

In the method now suggested, the distribution of moisture within individual grains is explored through examination of small particles dissected from specified points within the endosperm. The moisture content of a particle is assessed, to within $\pm 0.3\%$, through determination of its density which (over the useful range) is linearly related to moisture content, and is determined by the method of suspension in mixed non-aqueous liquids. The particle is protected by enclosure in a film of mineral oil from moisture change during dissection and transportation. Satisfactory application of the method depends, however, upon certain experimental precautions which are described in detail.

Many workers have determined the density of wheat by observing the displacement by the grain of a liquid such as xylene. The relation between moisture content and density of grain has been particularly investigated by Sharp (13), Brückner (3), and Matveef (8). The method of determining the density of individual small particles by suspension in mixed liquids appears to have been developed in 1889 by Retgers (11), who applied it to crystals of water-soluble salts. He claimed an accuracy of only one in the third decimal place but disregarded the effect of temperature variation. More recently (14) an accuracy of one in the fourth place has been obtained with individual crystals in the size range 0.1 — 2 mm. by means of close temperature control. Developments of the method have been applied, for example, by Hendricks and Jefferson (5) in optical studies, and by Bernal and Crowfoot (2) in X-ray investigations of molecular structure.

Neumann (9) early applied the method to determination of the density of grain by adjusting the composition of a mixture of carbon tetrachloride and petrol ether (ligroin) in which a sample was placed until half the grains, measured by bulk, sank. The method was recommended only for grain whose moisture content was in the range 12–15% (in atmospheric equilibrium). No investigation of variation in density of endosperm with moisture content has been traced in the literature.

Materials and Methods

Wheats. Table I shows a list of the wheats studied. Experiments were made on vitreous grains, hand-picked from each sample. Most of the wheats consisted mainly of vitreous grains but the Atle contained a large proportion of mealy or partly mealy grains. This explains why a marked difference in protein content between the original sample and the selected vitreous grains occurred in this case only. "Manitoba A" consisted of uniformly sized grain sifted from 1947 crop No. 1 Manitoba Northern wheat through No. 7 and over No. 8 wires (aperture widths 3.1 and 2.7 mm., respectively). It contained 80% of wholly vitreous grains, separated by hand-picking, which weighed 2.63 g. per 100 grains at 12.4% moisture content.

TABLE I

ORIGINS AND PROTEIN CONTENTS OF WHEATS

	Crop Year	Protein Content[1]	
		Original Sample	Vitreous Grains
'Manitoba A'	1947	13.4	13.1
No. 4 Manitoba northern[2]	1951	12.8	...
No. 1 Hard winter	1950	11.6	11.9
Atle, English-grown	1951	10.15	11.1
Russian	1951	13.2	13.5
Russian[3]	1931	12.8	...
Thatcher[4]	1947	15.3	15.15
Marquis[4]	1951	14.1	...
Red Bobs[4]	1947	13.5	...

[1] 14% moisture content basis.
[2] Of Atlantic shipment.
[3] Had been kept 20 years in a glass jar at laboratory temperature.
[4] Received by courtesy of Dr. J. A. Anderson, Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Manitoba.

Semolina (sizings or coarse middlings) was prepared from the wheat from which Manitoba A was drawn. The cleaned wheat, cold-conditioned to 16% moisture content, was passed through the first three breaks of the laboratory milling system, using rolls with 18 corrugations per inch. The collected grinds, scalped through No. 24 grit gauze and dressed over No. 60 grit gauze, were graded by sifting and freed from loose bran by treatment in a laboratory purifier.

Moisture Content Determination. In these laboratories moisture is determined by ascertaining the loss in weight on exposing semolina or coarsely crushed wheat for 4 hours at 120°C. in an electric oven. The wheat is crushed by means of a laboratory grinder with burred

discs (as in a coffee mill) so that not more than 20% of the grind passes through a No. 36 grit gauze sieve (aperture length 0.53 mm.). Under English atmospheric conditions evaporative loss during grinding is thus negligible even up to 20% moisture content and a one-stage moisture test is used. The test gives similar results to those by the A.A.C.C. method (1) in which wheat, ground so that 50% passes through No. 36 g.g., is heated for 1 hour at 130°C. In this paper all moisture values are on the moist basis.

Density Determination. The particle is placed in 25 ml. carbon tetrachloride contained in a stoppered graduated cylinder. Successive additions of a light liquid are made until, after mixing followed by three minutes' rest, the particle shows inappreciable movement vertically during a further two minutes. At first petroleum ether was used as the light liquid; later cyclohexane was preferred because its vapour pressure is nearer to that of carbon tetrachloride. Mixing must be thorough: as a standard procedure the cylinder was inverted ten times after each addition of cyclohexane. Two other essential conditions are:

a) temperature control to within 0.1°C. A water-filled thermostat with glass sides for convenience in observation was used. The cylinder was removed for shaking.

b) adequate closing of the cylinder. The cylinders (6¼"×1") had "E-mil" plastic stoppers which were found convenient.

Without these precautions slight convection currents occur, preventing establishment of the suspension point.

The cyclohexane was added from a micro burette whose tap was lightly lubricated with syrupy phosphoric acid. Densities (25°/25°) of the carbon tetrachloride and cyclohexane were 1.5898 and 0.7750 respectively. As an example, a particle of density 1.459 required for suspension the addition of 4.73 ml. cyclohexane; a further addition of 0.01 ml. caused it to sink. The density could be calculated from the volumes mixed but for greater accuracy a Sprengel-Ostwald pycnometer was filled with a sample of the liquid in which the particle was suspended; the usual precautions in handling mixed volatile liquids were taken during filling and weighing.

Determinations of suspension points and of liquid densities were all made at 25°C. At this temperature liquid viscosity is much reduced, observation of particle movement being correspondingly aided. All results refer to the density of water at 25°C; they may be reduced to 25°/4° by multiplication by 0.99704. The results have not been corrected for displacement of air during weighings, but may be re-

duced to *in vacuo* values by subtracting an amount ranging pro rata from 0.0008 at density 1.46 to 0.0006 at 1.40. This correction is based on the value 0.0012 g./ml. for the density of air. For present purposes the effect of variation in atmospheric density during weighings is negligibly small. This applies also to the presence of dissolved air in water and organic liquids.

Water Content of Organic Liquids. Water is slightly soluble in organic liquids with which it is immiscible; i.e., carbon tetrachloride at 25°C. dissolves about 0.01% by weight of water. This affects the present method by reason of the large difference in mass between a particle and the liquid in which it is suspended. If the liquid is dry its capacity causes the withdrawal of a considerable proportion of the moisture contained in the particle and a corresponding rise in its density. It is known that, within its small extent, the water content of an exposed non-miscible liquid responds to change in atmospheric humidity and it appears also to be related to the moisture content of wheat endosperm in contact with the liquid. Thus it was found that air-dry particles (12–14% moisture content) show equilibrium with liquids which also are in atmospheric equilibrium, while particles at 18% moisture content lose moisture to such liquids but not to those which have been water-saturated. Furthermore moist particles lose moisture rapidly while dry particles absorb it relatively slowly. Full discussion of this subject is outside the scope of this paper, but essentially both air-dry and water-saturated liquids showed practically the same density for a dry particle. Also, dry and moist particles were only slightly differentiated in density by air-dry liquids but were markedly differentiated by saturated liquids.

In applying the method, therefore, both the carbon tetrachloride and the cyclohexane were well shaken with water at room temperature, allowed to stand overnight, then separated by decantation. Care was taken to exclude suspended water droplets which would interfere if they were to touch a suspended particle. Liquids saturated at room temperature (17°C.) and at 25°C., respectively, gave similar results.

Titration End-point. Particles of about 12% moisture content behave well during titration. Towards the end-point their rate of movement depends upon the distance from that point. Once suspended they remain so for a long period, given constancy of temperature. The indicated density is independent of the direction of titration; back-titration may therefore be used if excess of light liquid has been added in the first approach.

With particles above about 16% in moisture content, on the other

hand, titrations in opposite directions give different results. Moreover the particles show instability: after brief suspension they sink, the rapidity of the change depending on the extent to which the composition of the liquid has been altered while in contact with them. This behavior may possibly be due to adsorption from the mixed liquid; it is known (10) that suspended powders may be affected through an adsorbed layer consisting of the liquid medium or of one of its constituents.

Satisfactory operation with moist particles was found possible, however, provided that the period of titration was minimized, and that the initial composition of the liquid mixture approximated that giving suspension. For example, a particle at 16.2% moisture content was placed in a mixture containing 25 ml. carbon tetrachloride and 5.30 ml. cyclohexane. The further portion-wise addition of 0.12 ml. cyclohexane required for suspension took 10 minutes. The particle was then stationary during the usual observational period (2 minutes) and was found to remain suspended for a total period of 15 minutes.

In practice these conditions imply either approximate fore-knowledge of the density or provision of two or three replicas of the particle, one being used for a preliminary titration and the others for closer approach. In the present field the first course is sometimes possible and the second usually so; there is no difficulty during dissection in separating three neighboring fragments from a given region of the grain.

Preparation of Particles (Dissection of Grain). A few grains from the sample are dropped into light mineral oil (medicinal liquid paraffin). The selected grain is removed from the oil and gripped in a pin vice held vertically with its stem inserted through a hole in a platform beneath a binocular magnifier. After the grain has been sectioned transversely (horizontally) with an oil-covered blade, the exposed surface is viewed through the magnifier and two or three thin slices removed with a sharp oil-coated safety razor blade, in order to clean the surface and leave it completely vitreous. The chosen zone (in the present tests, about 0.5 mm. square) is then isolated, by means of a series of nearly vertical incisions made around it. By suitably inclining the blade during the operation, the surrounding endosperm may readily be chipped away in relatively large blocks without disturbing the chosen region. This is then detached by inserting horizontally a needle whose end has been ground, under magnification, to a chisel edge. In practice the oil remains as a copious covering on the surface of the section and automatically surrounds the particle

during excision. The oil-covered particle readily adheres to the needle by means of which it may be transferred to the liquid in the cylinder.

In illustration of the effect of the oil, particles dissected, with and without oil respectively, from grain at 18% moisture content had densities 1.436 and 1.454, while particles from grain in atmospheric equilibrium showed the same value, 1.456, whether dissected with or without oil. Thus: 1) without oil, particles from moist grain change almost to the air-dry level; 2) apart from preventing loss of moisture, the oil itself has no effect on the determination.

The oil used in these experiments was in moisture equilibrium with the laboratory air whose average relative humidity was about 50–55%. Similar results, both on air-dry and moist particles, were obtained using oil which had been water-saturated. On the other hand, oil which had been previously dried over calcium chloride caused marked increase in density in particles from moist grain, evidently as a result of dehydration. If the present method were used under very dry atmospheric conditions, therefore, it would be desirable to use oil which had been water-saturated.

Adjustment of Moisture Content. In order to ensure uniform damping, Manitoba A was graded in grain size as previously described and wetted, not by sprinkling, but by immersion for varying short periods followed by centrifuging (6).

Individual particles were adjusted in moisture content through exposure, usually in small groups, on watch glasses placed in a narrow circle near the center of a vessel of the type ordinarily used as desiccators. Sulfuric acid of suitable strength was contained in the lower part of the vessel. It was also placed in a shallow dish supported near the top so that the samples under examination were exposed between surfaces of the humidity-controlling agent. The desiccators were placed in a constant temperature chamber at 20°C. As controls, three weighed samples of semolina of known initial moisture content were spread in shallow aluminum containers placed closely surrounding the watch glasses bearing the specimens. Two of the samples, consisting of particles slightly larger than the specimens, differed in weight, one being spread much more thinly than the other. The particles forming the third sample were rather smaller than the specimens. After exposure the aluminum containers were covered with well-fitting lids and weighed, the moisture content of the semolina being calculated from the change in weight.

With adequate temperature control and sufficient duration of exposure (5 days at 20°C.), all three control samples showed closely simi-

lar proportional increases in weight. This was accepted as guaranteeing 1) uniformity of conditions throughout the vessel, 2) duration of exposure sufficient to ensure correspondence in proportional weight increment between specimens and control samples. Thus the individual particles were assumed to have reached the moisture content of the control semolina samples.

A rotatable tube, inserted through a bung in the lid tubulure and bent to pass the acid dish, enabled the particles in the watch glasses to be covered with oil just before the vessel was opened. After this treatment replicate particles at 18% moisture content showed no appreciable change in density during 30 minutes' exposure to the laboratory atmosphere, whereas similar particles without oil dried so rapidly that their density increased by 0.006 during transference to the suspension liquid.

Results

Variability of Endosperm Density at a Given Moisture Content. (a) Between Grains of a Given Sample. The feasibility of the present method depends upon a sufficiently limited spread in density between particles taken from corresponding positions in different grains of a sample at a given moisture content. Experience has shown that in fact, with vitreous grains, the spread is more limited than was expected. Thus with Manitoba A wheat (see Materials and Methods), at its "natural" moisture content of 11.8%, particles dissected from the center of the cheek of each of five vitreous grains taken at random, showed densities ranging from 1.4608 to 1.4614, mean 1.4612.

Naturally, greater variation between grains is shown in the case of wetted wheat, even with the precautions to ensure uniformity of wetting described under "Materials and Methods." Thus the following observations relate to central cheek particles from five grains taken at random from Manitoba A after adjustment to 17.6% moisture content. They are given in extenso, with descriptions of the grains, since uniformly vitreous grains change on damping, showing varying degrees of opacity: a feature likely, a priori, to influence density.

Appearance of Grain		*Density of Endosperm*
External	*Sectioned*	
Unusually 'bleached'	Vitreous	1.4371
Normally 'bleached'	Slightly mealy	1.4385
Normally 'bleached'	More mealy than 2	1.4381
Normally 'bleached'	Quite mealy	1.4356
Normally 'bleached'	Quite mealy	1.4362

The range is 1.4356 to 1.4385, mean 1.4371. Perhaps surprisingly, the density is not affected by incidence of slight mealiness (a feature to which reference will be made in a further paper), and if the grains found on sectioning to be quite mealy are excluded the range of density is much narrowed. Thus the densities of central cheek particles from ten non-mealy grains from Manitoba A at 17.1% moisture content ranged from 1.4383 to 1.4403, mean 1.4396. By examining particles from at least three such grains the moisture content may be inferred with an error within $\pm 0.3\%$.

(b) Between Grains of Different Types of Hard, or Semi-hard, Wheat. Figure 1 (drawn on a basis described in the section on moisture content) shows that, at similar moisture contents, the density of the central cheek endosperm of vitreous grains of the following wheats was within 0.0015 of that of Manitoba A: Red Bobs, Thatcher, No. 4 Manitoba Northern, No. 1 Hard Winter, Russian (2 samples), Atle. The first three are Canadian wheats; the last is a relatively hard type of English-grown wheat, bred by Mr. Weibulls of Sweden. With

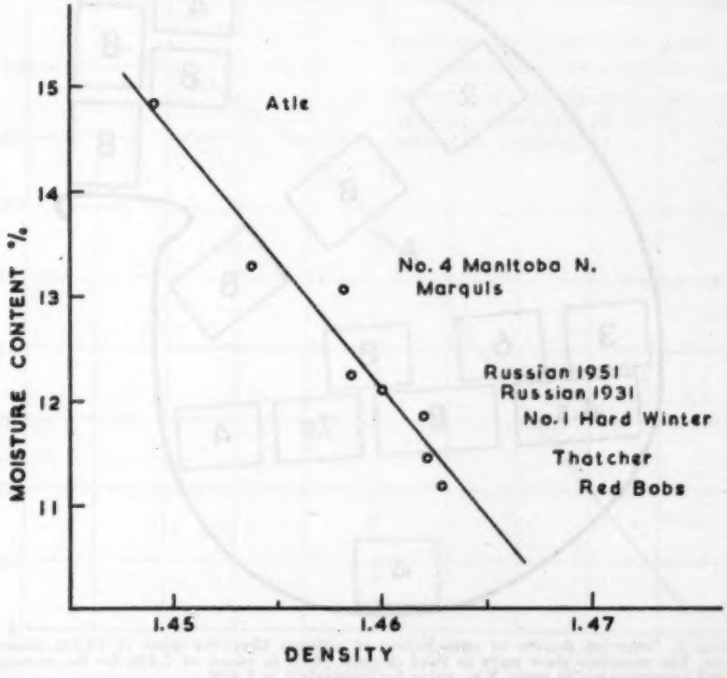

Fig. 1. Density of central endosperm in vitreous grain of different wheats. The continuous line (from Fig. 3) shows relation between moisture content and density of endosperm for Manitoba A.

the remaining sample, Marquis, the density was within 0.0025 of that of Manitoba A. Figure 1 and Table I together suggest that variation in protein content of wheat does not affect the density of its endosperm (provided it is vitreous).

(c) Within Individual Grains. Figure 2 shows the origins in a transverse section, midway along a grain of Manitoba A at 12.6% moisture content, of particles whose densities were as shown in the legend.

The density was uniform over a large region but fell from the center to the outer portion of endosperm, by 0.005 in the cheek and by 0.008 in the dorsal region. Examination of longitudinal sections showed that the density of the endosperm was uniform at corresponding points in the cross-section over two-thirds of the distance from the germ end but fell by about 0.005 towards the beard end.

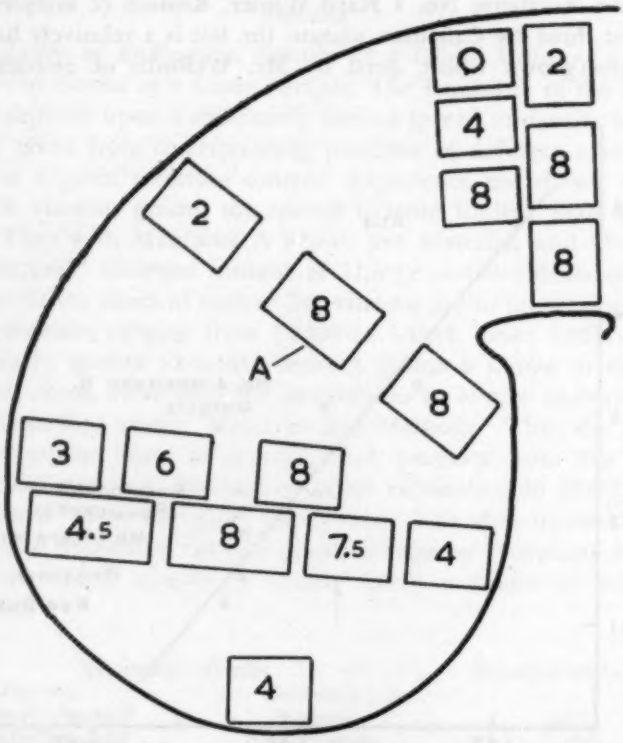

Fig. 2. Range of density in cross-section of vitreous Manitoba grain at 12.6% moisture content. The numerals show units in third decimal place in excess of 1.450 for the rectangles outlined approximately to scale. E.g., value for rectangle A is 1.458.

Variation of Endosperm Density With Moisture Content. Figure 3 shows the relation between moisture content and density for:

(i) endosperm particles dissected from vitreous grains at various moisture contents.

(ii) particles moistened, by controlled vapour uptake, after dissection from the cheeks of grains at about 12% moisture content.

The points for (i) and (ii) both fall on a line showing direct proportionality between density and moisture content in the range 10-20%. The gradient is 0.0041 in density per 1% difference in moisture content. Above 20% the line bends over, showing acceleration in density fall with increasing moisture content. Particles dissected from the dorsal part of the grain gave a line having the same slope as that shown in Fig. 3. Although the systematic calibration shown in Fig. 3 applies only to one wheat (Manitoba A), Fig. 1 gives indirect evidence that it would be similar for other wheats. It applies however only to particles from grains which are vitreous at about 12% moisture content; the subject of mealiness will be discussed in a later paper.

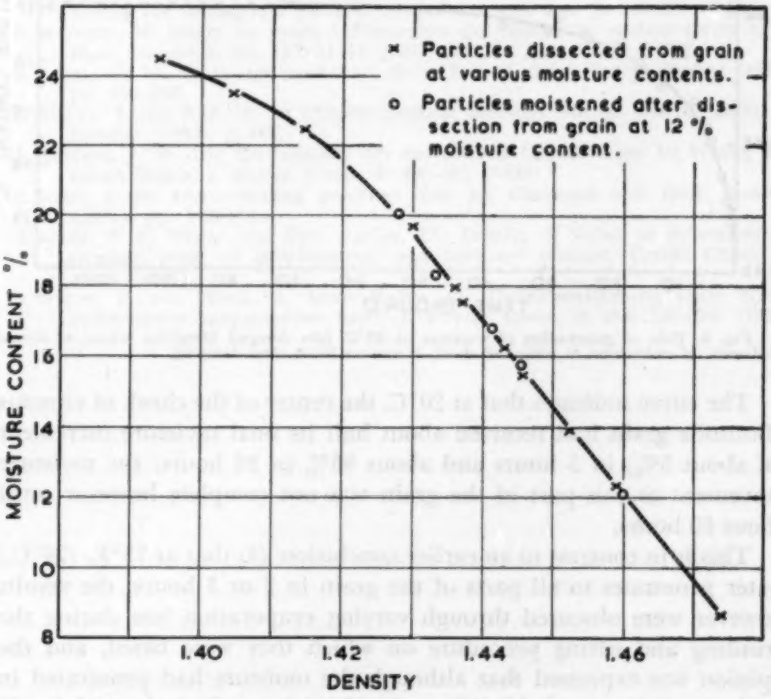

Fig. 3. Relation between density and moisture content of endosperm of vitreous grains.

An Application of the Method: The Rate of Penetration of Moisture at 20°C. to the Center of the Cheek in Damped Manitoba Wheat. A sample of Manitoba A wheat at 12.4% moisture content was well stirred under water at 20°C. for 1 minute, then centrifuged and stored in small air-tight jars in a constant temperature chamber at 20°C., its moisture content (by oven determination) being 17.2%. At intervals, central cheek particles were dissected from each of three representative vitreous grains and their densities determined. The average results are shown in Fig. 4, which also shows the corresponding moisture contents derived from Fig. 3. None of the individual points is further than the equivalent of about 0.25% in moisture content from the continuous curve. The discontinuous horizontal line shows the density of central cheek endosperm found in grain of 17.2% moisture content which had stood in air-tight containers for an indefinitely long period.

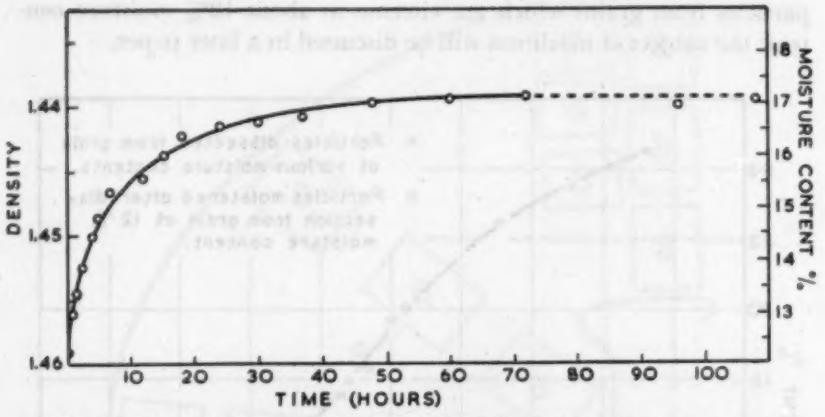

Fig. 4. Rate of penetration of moisture at 20°C. into damped Manitoba wheat as shown by density of endosperm in center of cheek at various times after damping.

The curve indicates that at 20°C. the center of the cheek of vitreous Manitoba grain had received about half its final moisture increment (of about 5%) in 5 hours and about 85% in 24 hours; the moisture movement to this part of the grain was not complete however until about 60 hours.

This is in contrast to an earlier conclusion (4) that at 75°F. (24°C.) water penetrates to all parts of the grain in 2 or 3 hours; the results however were obscured through varying evaporation loss during the grinding and sifting procedure on which they were based, and the opinion was expressed that although the moisture had penetrated in 2-3 hours it became held differently when the grain had stood for 23

hours. Results obtained by Scott (12), also by means of a grinding and sifting method, suggested that moisture was still moving after 7 hours but was substantially stationary at 26 hours at ordinary temperature. Other methods of approach have suggested little change in moisture distribution after 7 hours (7) but, by their nature, they probably reflect only the departure of moisture from the bran into the endosperm as a whole.

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SUSCEPTIBILITY TO HYDROLYSIS BY BETA-AMYLASE OF THE SOLUBLE AND INSOLUBLE FRACTIONS OF THE CRUMB OF CONVENTIONAL AND BACTERIAL ALPHA-AMYLASE SUPPLEMENTED WHITE BREAD[1]

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ABSTRACT

The distribution and behavior of the soluble and insoluble starch fractions of bread crumb was studied, in terms of susceptibility to hydrolysis by beta-amylase, in white breads baked with and without the addition of bacterial alpha-amylase. The amount of susceptible starch in the aqueous extract of conventional white bread was one-tenth or less that in the insoluble fraction and remained essentially constant despite prolonged storage at 8°, 30°, and 50°C. In contrast, the amount of susceptible starch in the insoluble fraction decreased as the bread aged, the rate and extent of decrease showing the characteristic dependence of the staling process on temperature. It would thus appear that the soluble starch fraction of bread crumb is incapable of appreciable retrogradation, as evaluated by decreased susceptibility to beta-amylase, and that the phenomenon of crumb starch staling is confined primarily to the insoluble part of the crumb. In the case of bread baked with bacterial alpha-amylase there was a significant increase during storage, at the expense of the insoluble fraction, in the amount of soluble starch susceptible to beta-amylase. These and other data were interpreted to indicate that the residual alpha-amylase continued to function during storage of the bread.

Although the mechanism of bread staling is not clearly understood considerable evidence has accumulated indicating that changes in the starch fraction are of major importance in the staling phenomenon. It is generally accepted (6, 11, 19) that the starch changes are "retrogradative" or "associative" in nature representing partial reversion towards the non-gelatinized condition. Recent data (7) obtained by an improved fermentation technique indicated, in confirmation and extension of earlier reports (10, 16), that the susceptibility of crumb starch to enzymatic hydrolysis by beta-amylase decreased progressively with increasing staleness of the bread. The discovery that the decrease in susceptibility of the starch during aging of bread could be prevented or retarded by the inclusion of small amounts of bacterial alpha-amylase in the bread formulation was also reported recently (17).

The present investigation was undertaken to ascertain whether the

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decrease in susceptibility of crumb starch to beta-amylase during staling is localized in the soluble or insoluble fraction of the crumb, and to determine whether any differences in this respect exist between conventional white bread and bread baked with bacterial alpha-amylase. It was anticipated that the information obtained from such a study would be of value in providing further data relative to the disagreement between reports indicating a progressive decrease in soluble starch on staling (3, 5, 5a, 9, 12, 15, 20) and other reports questioning both the accuracy and significance of soluble starch measurements with reference to the staling phenomenon (1, 2, 4, 8).

Materials and Methods

Bread. White breads were prepared on a commercial scale in 50 lb. flour batches by the sponge dough process. The basic formula has been given previously (7). Breads supplemented with bacterial alpha-amylase were baked by the basic formula with addition in the sponge stage of a purified bacterial (*B. subtilis* type) alpha-amylase enzyme in an amount equivalent to 3 or 6 Sandstedt, Kneen, and Blish units (14) of alpha-amylase per pound of bread. Characteristics of the enzyme supplemented breads[3] have been described previously (17). The baked loaves were sealed in moisture-proof cellophane bags after reaching room temperature and stored at the specified temperatures for future testing.

Preparation of Soluble Extract. Water-soluble extracts of the breads were prepared immediately before testing by weighing 40 g. of interior bread crumb into a 250 ml. centrifuge bottle, adding 100 ml. of water at about 25°C., and dispersing the wetted crumb by stirring for 2 minutes with an electric stirrer (Hamilton-Beach) equipped with a button type impellor. The insoluble material was settled by centrifuging at 2000 r.p.m. for 10 minutes and the supernatant (volume about 65 ml.) was collected by decantation. The residue was dispersed in a second 100 ml. portion of water and the supernatant resulting from centrifuging was combined with the first decantate. The combined supernatants (volume about 165 ml.) were diluted to 200 ml. with water, mixed, and filtered through folded Whatman No. 12 filter paper. The opalescent filtrates were used for subsequent measurement, 100 ml. aliquots representing the soluble extract from 20 g. of crumb.

Measurement of Susceptibility to Beta-Amylase. The method used for determining susceptibility of the crumb to attack by beta-amylase

[3] Subsequent to the writing of this paper there was issued a patent (18) concerned with the use of bacterial alpha-amylase in baked goods.

has been reported previously (7). This method was used to determine susceptibility of the soluble starch by transferring a 100 ml. aliquot of the soluble extract solution to a 250 ml. reaction bottle, adding the buffer, thiamine, and yeast, and proceeding as directed in the published procedure including the fermenting out period and the subsequent beta-amylase conversion. Fermentation and conversion were generally complete in 1 hour.

Results and Discussion

Conventional White Bread. It was reported previously (7) that the crumb starch of conventional white bread decreased significantly in susceptibility to enzymatic hydrolysis by beta-amylase as the bread staled. The data of Table I, representing susceptibility to beta-amylase of both the entire crumbs and their water soluble extracts, were obtained in order to establish whether the changes on aging are localized primarily in the soluble or insoluble fractions of the crumb. The results make it evident that the soluble fraction is not involved significantly in the staling changes which are measured by susceptibility to beta-amylase.

TABLE I

INFLUENCE OF TIME AND TEMPERATURE OF STORAGE ON SUSCEPTIBILITY OF CRUMB STARCH IN CONVENTIONAL WHITE BREAD TO BETA-AMYLASE

Time of Storage	Gas Production in Fermentation Test[1]					
	8°C. Storage		30°C. Storage		50°C. Storage	
	Entire Crumb	Extract	Entire Crumb	Extract	Entire Crumb	Extract
hrs.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
0	611	27	611	27	611	27
17	320	20	509	25	538	27
41	222	27	410	22	525	23
65	200	25	347	25	505	25
89	187	20	315	21	455	25

[1] The data reported are the amounts of gas produced in two hours from 20 g. of bread crumb stored at the indicated temperature or from the water soluble extract of 20 g. of bread crumb stored at the indicated temperature after conversion with 35 mg. of 2000° Lintner beta-amylase.

Thus, the gas production from the extracts accounted for not more than approximately 5 to 10% of the total volumes of gas realized from the entire crumb. This implies that at least 90% of the starch

susceptible to beta-amylase is not water soluble even when the bread is oven-fresh and appears to raise a serious question about the significance of soluble starch in the staling phenomenon.

Further argument against the involvement of the soluble fraction in staling may be found in the constancy of the gas production from the extracts regardless of the time or temperature of storage. Thus, the volumes of gas produced from the extracts remained constant at about 25 ml. under conditions where gas production from the crumbs decreased from 611 to 187 ml. Similarly, the extracts remained constant at 25 ml. regardless of the temperature of storage, whereas the susceptibility of the crumbs decreased further and more rapidly as the storage temperature was lowered. Since the behavior of the crumb in relation to temperature of storage, but not of the extract, is in agreement with other reports (4, 7, 13, 16) indicating that bread stales faster as the temperature is reduced, it appears reasonable to conclude that the insoluble, but not the soluble, starch fraction is of major importance in the staling phenomenon.

To validate the conclusion that the decrease in susceptibility to beta-amylase accompanying staling is confined primarily to the insoluble starch fraction, it is necessary to establish that the extraction procedure used was exhaustive, that gas production from the insoluble residue was essentially equal to that of the entire crumb, and that white bread baked in other bakeries displayed the same type of behavior.

Preliminary evidence indicating that the extraction conditions employed were satisfactory was obtained from experiments in which the effect of larger volumes of water and longer extraction periods were studied. Identical results (25 ± 5 ml. of gas from the soluble extract of 20 g. of crumb) were obtained regardless of the length of the extraction period (2 minutes to 4 hours) or the volume of extractant (100 or 200 ml. of water per 40 g. of crumb).

Other data bearing on the question of efficiency of the extraction procedure were obtained by comparing gas productions of the crumbs and of their water extracts during the fermenting out period, prior to beta-amylase addition. These data, which are a measure of fermentable carbohydrates (primarily mono- and di-saccharides) are given in Table II. Although the temperature of storage had some influence on the extraction process, the average efficiency at the temperatures employed was in the range of 70 to 85%. (Similar efficiencies of extraction were also found for white breads baked with 6 Sandstedt, Kneen, and Blish units of bacterial alpha-amylase.) The theoretical efficiency for an ex-

traction of this type may be calculated[4] as 85% if complete release of soluble components is assumed. It appears reasonable to consider the extractability of the fermentable carbohydrates as an index of the extractability of the soluble starch. It may thus be concluded that the extraction procedure was sufficiently exhaustive to be valid for the purposes of this study since the soluble extract data of Table I will not be in error, in terms of incomplete extraction, by more than 5 to 10 ml. of gas.

TABLE II

COMPARISON OF GAS PRODUCTION DURING FERMENTING OUT PERIOD FROM THE CRUMB AND SOLUBLE EXTRACT OF CONVENTIONAL WHITE BREAD

Time of Storage	Gas Production Prior to Beta-Amylase Addition[1]								
	8°C. Storage			30°C. Storage			50°C. Storage		
	Entire Crumb	Extract	Efficiency	Entire Crumb	Extract	Efficiency	Entire Crumb	Extract	Efficiency
hrs.	*ml.*	*ml.*	*%*	*ml.*	*ml.*	*%*	*ml.*	*ml.*	*%*
0	117	81	69	117	81	69	117	81	69
17	123	115	94	133	93	70	121	88	73
41	133	121	91	133	105	79	138	91	66
65	135	116	86	134	110	82	143	107	75
89	135	117	87	140	107	76	145	97	67
Average	..	..	85	..	..	75	..	..	70

[1] The data reported are the total amounts of gas produced from 20 g. of bread crumb stored at the indicated temperature or from the water soluble extract of 20 g. of bread crumb stored at the indicated temperature prior to beta-amylase conversion.

Evidence indicating that the extraction procedure did not alter the nature of the starch with consequent effect on the susceptibility to hydrolysis by beta-amylase is presented in Table III. Since approximately 90% of the attackable starch in the crumb was recovered in the insoluble fraction remaining after water extraction, it may be concluded that the soluble extract data of Table I are valid and correctly indicate that only a small amount (about 10%) of the starch involved in the staling phenomenon is soluble.

The general nature of the conclusions drawn from experiments with conventional white bread baked in our own bakery unit was established by measurements taken on commercial white breads purchased periodically on the open market during 1951. Typical data on

[4] Calculated by using data given in **Materials and Methods** section indicating recovery of 65 ml. of solution from the first wash of the 40 g. crumb sample with 100 ml. of water and recovery of 100 ml. of solution from the second wash with 100 ml. of water. Assuming 40% moisture in the crumb, and taking into account the holdback by the crumb of an additional 35 ml. of water, maximum recoveries may be estimated at 56% in the first wash and an additional 29% in the second wash.

the same brands of bread purchased in July and November, 1951 are presented in Table IV. The breads were analyzed immediately after purchase, which was timed to coincide with the first delivery of the day, and after 72 hours storage at 30°C.

TABLE III

COMPARISON OF SUSCEPTIBILITY OF THE ENTIRE CRUMB AND INSOLUBLE CRUMB FRACTION OF CONVENTIONAL WHITE BREAD TO BETA-AMYLASE

Time of Storage at 30°C.	Gas Production in Fermentation Test[1]			
	Entire Crumb	Extract	Insoluble Residue	Residue / Crumb
hrs.	*ml.*	*ml.*	*ml.*	%
17	513	24	473	92
41	442	23	414	94
106	246	25	205	83
130	230	24	213	93
Average	..	..	..	90.5

[1] The data reported are the amounts of gas produced in two hours from 20 g. of bread crumb or from the insoluble residue corresponding to 20 g. of bread crumb after conversion with 35 mg. of 2000° Lintner beta-amylase.

TABLE IV

SUSCEPTIBILITY OF CRUMB STARCH IN COMMERCIAL SLICED WHITE BREAD TO BETA-AMYLASE

Brand	Gas Production in Fermentation Test[1]							
	Entire Crumb				Extract of Crumb			
	July, 1951		November, 1951		July, 1951		November, 1951	
	As Received	Stored[2]	As Received	Stored[2]	As Received	Stored[2]	As Received	Stored[2]
	ml.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
A	420	250	236	137	20	22	25	23
B	395	240	534	245	20	28	23	15
C	431	236	192	85	15	15	22	20
D	350	189	515	177	12	11	9	10
E	449	308	305	207	15	15	31	28
F	475	266	..	..	16	15	..	..
G	..	..	432	135	..	..	25	26
Average	420	248	369	164	16	18	22	20

[1] See Table I, Footnote 1.
[2] Stored for 72 hours at 30°C.

There was wide variability among the breads with respect to susceptibility of the crumbs to attack by beta-amylase. Although no con-

clusions can be drawn about the freshness of the breads on receipt, since there was no assurance that the breads were freshly baked, it is apparent that each of the breads decreased appreciably in susceptibility as they staled during the 72 hour storage period. In agreement with the data of Table I, gas production from the soluble extract amounted to only 5 to 10% of that from the entire crumb. In further agreement with the previous data there was no evidence of decrease in susceptibility of the polysaccharides in the soluble extract during the storage period. It appears, therefore, that the conclusion drawn previously indicating that the insoluble starch fraction, but not the soluble extract, is primarily involved in the decrease in susceptibility to beta-amylase, is valid and general in nature.

Further definite confirmation of the thesis that starch staling is confined to the insoluble crumb fraction was obtained by experiments in which breads stored at 30°C. and 50°C. for 89 hours were transferred to a refrigerator and stored for an additional 72 hours at 8°C. The data of Table V establish that the amounts of soluble starch susceptible to hydrolysis by beta-amylase were not reduced by the additional storage at low temperature. Since the temperature employed, 8°C., is known to accelerate staling it appears reasonable to conclude that the polysaccharides in the soluble extracts were sufficiently degraded to be incapable of decreasing in susceptibility to attack by beta-amylase. For comparison, it should be noted that the amounts of attackable starch in the entire crumbs were reduced sharply by the further refrigerator storage, susceptibility decreasing by approximately 50%. This finding, coupled with the data indicating no change in the soluble extract, tends to confirm the thesis that the decreased susceptibiltity of the crumb to beta-amylase during staling results from changes in the insoluble, rather than soluble, crumb fractions.

The results obtained in this study indicate that the soluble starch fraction is not involved significantly in the decreased susceptibility of the crumb to beta-amylase during staling. In this respect they are in complete agreement with Bechtel and Meisner (1) who recently concluded that changes in soluble starch, whether determined as total soluble carbohydrates, soluble amylose, or soluble amylopectin, could not be related to the staling process.

The present results indicate further that the changes responsible for the decreased susceptibility of stale bread crumb are localized primarily in the insoluble starch fraction. This conclusion may be disturbing at first glance since it requires acceptance of the not sufficiently well realized concept that enzymatic reactions may take place in the

TABLE V

EFFECT OF LOW TEMPERATURE STORAGE FOLLOWING HIGH TEMPERATURE STORAGE ON SUSCEPTIBILITY OF CRUMB STARCH TO BETA-AMYLASE IN CONVENTIONAL WHITE BREAD

Storage Conditions	Gas Production in Fermentation Test[1]	
	Entire Crumb	Extract
	ml.	*ml.*
89 hrs. at 50°C.	455	25
89 hrs. at 50°C. + 72 hrs. at 8°C.	219	26
Difference	−236	+ 1
89 hrs. at 30°C.	315	21
89 hrs. at 30°C. + 72 hrs. at 8°C.	187	20
Difference	−128	− 1

[1] See Table I, Footnote 1.

presence of *insoluble* substrates. Typical examples of common reactions involving *insoluble* substrates are plentiful and include, for instance, the tenderizing of meat by papain and the use of proteinases to solubilize and degrade coagulated proteins. The data of this paper provide evidence that beta-amylase can hydrolyze the *insoluble* starch in bread crumb to soluble, fermentable moieties.

Bacterial Alpha-Amylase Supplemented Bread. It has been reported recently (17) that the inclusion in the formulation of 3 to 6 Sandstedt, Kneen, and Blish units of bacterial alpha-amylase per pound of bread resulted in white bread normal in all respects but characterized by a greatly lessened rate of change during aging in susceptibility of the crumb starch to beta-amylase. It appeared of interest to determine whether the soluble extract of bread made with a formula containing bacterial alpha-amylase follows the same pattern on aging as conventional white bread. It was expected that differences might exist since the enzyme treated breads differ from conventional products in that susceptibilities of the crumbs to beta-amylase are maintained at high levels, characteristic of fresh bread, over prolonged storage.

The data of Table VI, representing susceptibilities of both entire crumbs and their water soluble extracts to beta-amylase, make it evident that these breads do not age in the same way as conventional bread. Susceptibility of the crumb decreased appreciably during prolonged storage at 8°C., only very slightly during similar storage at 30°C., and actually increased significantly when the breads were stored at 50°C. Correspondingly, the extracts showed little change during

storage at 8°C. but progressively large increases in the amounts of susceptible starch during storage at 30°C. or 50°C. Thus, gas production from the crumb decreased somewhat over prolonged storage at 30°C. from 659 to 567 ml., whereas gas production from the extracts of the crumbs increased from 70 to 198 ml. The magnitude and nature of the difference between the enzyme supplemented and conventional breads may be realized when these data are compared with those of Table I for conventional bread. Under similar storage conditions, crumb gas production of the conventional product decreased sharply from 611 to 315 ml. whereas gas evolution from the crumb extract remained constant at about 25 ml.

TABLE VI

INFLUENCE OF TIME AND TEMPERATURE OF STORAGE ON SUSCEPTIBILITY TO BETA-AMYLASE OF CRUMB STARCH IN WHITE BREAD BAKED WITH 6 SKB UNITS OF BACTERIAL ALPHA-AMYLASE

Time of Storage	Gas Production in Fermentation Test[1]					
	8°C. Storage		30°C. Storage		50°C. Storage	
	Entire Crumb	Extract	Entire Crumb	Extract	Entire Crumb	Extract
hrs.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
0	659	70	659	70	659	70
17	410	75	545	135	600	215
41	332	58	550	173	745	205
65	295	53	560	183	750	256
89	298	50	567	198	757	226

[1] See Table I, Footnote 1.

An understanding of the origin of the increased amounts of susceptible starch appearing during storage in the soluble extracts of the enzyme treated breads may be obtained from the data of Table VII taken with bread baked with 3 Sandstedt, Kneen, and Blish units of bacterial alpha-amylase. The increased volumes of gas evolved from the soluble extracts are compensated for by corresponding decreases in the gas evolutions produced from the insoluble residues. As shown in the last column of Table VII, agreement between the experimental measurements of gas evolution from the insoluble residues and similar values calculated by difference between the crumbs and their soluble extracts are all in the range of 94 to 99%. It may thus be concluded that during storage of enzyme-supplemented breads there is a progressive conversion of insoluble starch moieties to forms which are water-

soluble. The most likely explanation at present, in view of previous data (17) showing that approximately 20% of bacterial alpha-amylase resists inactivation during baking, is that the residual alpha-amylase continues to function during storage to degrade the insoluble starch to soluble, lower molecular-weight entities.

TABLE VII

COMPARISON OF SUSCEPTIBILITY TO BETA-AMYLASE OF ENTIRE CRUMB, EXTRACT, AND INSOLUBLE CRUMB FRACTION OF WHITE BREAD BAKED WITH 3 SKB UNITS OF BACTERIAL ALPHA-AMYLASE

Time of Storage at 30°C.	Gas Production in Fermentation Test[1]				
	Entire Crumb	Extract	Insoluble Residue		
			Calculated[2]	Measured	Measured / Calculated
hrs.	*ml.*	*ml.*	*ml.*	*ml.*	%
17	559	22	537	530	99
41	510	50	460	430	94
106	470	109	361	340	94
130	455	106	349	330	95

[1] The data reported are the amounts of gas produced in two hours from 20 g. of bread crumb, or from the aqueous extract of 20 g. of bread crumb, or from the insoluble residue corresponding to 20 g. of bread crumb after conversion with 35 mg. of 2000° Lintner beta-amylase.

[2] Calculated by difference between the value for the entire crumb and the value for the soluble extract.

Information concerning the nature of the polysaccharides appearing in the soluble extracts of stored bread was obtained by experiments in which breads kept at 30°C. and 50°C. for 89 hours were transferred to the refrigerator and stored for an additional 72 hours at 8°C. It is evident from Table VIII that the susceptibility of the soluble polysaccharides was not reduced by the additional storage at low temperature. Since the temperature employed, 8°C., is known to accelerate staling it appears reasonable to conclude that the polysaccharides in the soluble extracts are sufficiently degraded to be incapable of decreasing in susceptibility to beta-amylase. It is significant that a similar conclusion was reached with respect to the soluble polysaccharides of conventional white bread (Table V). It would thus appear that the bulk of the polysaccharides appearing in the soluble extracts of bread are of sufficiently low molecular-weight to be incapable of associating, confirming further the thesis that the decreased susceptibility of the crumb to beta-amylase during staling results from changes in the insoluble, rather than soluble, starch fractions.

TABLE VIII

EFFECT OF LOW TEMPERATURE STORAGE FOLLOWING HIGH TEMPERATURE STORAGE ON SUSCEPTIBILITY OF CRUMB STARCH TO BETA-AMYLASE IN WHITE BREAD BAKED WITH 6 SKB UNITS OF BACTERIAL ALPHA-AMYLASE

Storage Conditions	Gas Production in Fermentation Test[1]	
	Entire Crumb	Extract
	ml.	*ml.*
89 hrs. at 50°C.	757	226
89 hrs. at 50°C. + 72 hrs. at 8°C.	737	225
Difference	− 20	− 1
89 hrs. at 30°C.	567	198
89 hrs. at 30°C. + 72 hrs. at 8°C.	563	202
Difference	− 4	+ 4

[1] See Table I, Footnote 1.

Further information concerning the mode of action of the bacterial alpha-amylase in enzyme-supplemented breads may be obtained from Table VIII. In contrast to the behavior of conventional breads, susceptibility of the crumbs of enzyme-treated loaves to beta-amylase did not decrease during the additional storage in the refrigerator. This behavior may be interpreted, in view of the data of Table VI indicating that the effectiveness of the alpha-amylase was greatly inhibited at 8°C., as suggesting that during storage at the higher temperature the enzyme degraded the starch to forms which were incapable of retrograding when exposed to the lower temperature. It would thus appear that the residual enzyme continued to function during storage to degrade some of the starch to moieties which were incapable of retrograding. The degraded molecules apparently vary in size from configurations which are too large to be water-soluble down to configurations which are small enough to be soluble but which are still large enough to require further degradation by beta-amylase in order to become fermentable by yeast.

Acknowledgment

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PHYSICAL PROPERTIES OF WEATHERED WHEAT IN RELATION TO INTERNAL FISSURING DETECTED RADIOGRAPHICALLY[1]

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ABSTRACT

The reduction in density of wheat caused by natural weathering or by wetting and drying in the laboratory is due to the formation of internal fissures which can be detected radiographically. These fissures appear when moisture-swollen grain is dried in a manner which will produce stresses within the wheat kernel. Rapid drying of immature grain of high moisture content does not produce fissuring, but such dried grain when rewetted and redried fissures readily, indicating a fundamental change in structure of the endosperm constituents with maturation.

Wheat hardness and the energy requirement for grinding are both reduced when grain becomes weathered and internally fissured. The rate of absorption of water is increased also. The practical inferences of these findings are discussed.

The weathering or bleaching of unharvested wheat in the field due to wetting by rain or snow, followed by atmospheric drying, involves an irreversible decrease in density of the grain. This phenomenon was investigated by Sharp (5) who suggested that internal air spaces are formed in the kernels when moisture-swollen grain is dried. Swanson (6, 7) showed that vitreous grain subjected to artificial weathering by wetting and drying, assumes the characteristics of field-weathered wheat but that the loss in the original vitreous condition accompanying the decrease in density of the grain was not reflected in a deterioration in the milling and baking qualities. In fact, in some cases an improvement in these technological properties occurred.

The discovery in this laboratory, utilizing X-ray technics, of internal fissures in weathered wheat oriented at right angles to the longitudinal axis of the kernel (3) afforded for the first time visual proof of the existence of internal spaces in weathered wheat as postulated by both Sharp and Swanson. This observation prompted additional laboratory studies designed to clarify the mechanism of fissure formation in grain when cycles of wetting and drying similar to those which might occur in the field were applied. It was also of interest to determine the influence of such internal fissuring on certain technological properties of the grain.

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Materials and Methods

The discovery that internal fissuring was related to weathering was made with a series of 20 samples of seed wheat obtained from the Kansas Crop Improvement Association, Manhattan, Kansas. In this group it was observed that internal fissures existed only in grain which was visually weathered and bleached. Subsequently the opportunity was provided to examine radiographically a number of commercial samples of hard red winter and hard red spring wheats which had been subjected to natural weathering[2]. By numerous inspections of this kind the relationship between internal fissuring and weathering was adequately confirmed.

Laboratory studies involving wetting and drying of grain were conducted with samples of hard red winter wheat including the varieties Pawnee and Ponca. The effect of rapid drying of immature wheat was investigated with samples of Pawnee wheat harvested from a plot on the Agronomy Farm of the Kansas Agricultural Experiment Station. A thermostatically controlled air oven with forced air circulation was used in the drying studies. Conditioning of the grain to various moisture contents was achieved by wetting the dry wheat in sealed containers with the required quantity of water and agitating several times over a 24 hour period. Moisture values were determined by means of the 130°C. air oven method (Cereal Laboratory Methods) and are expressed on the "as is" basis. The two-stage procedure was used for samples containing in excess of 12% moisture.

Radiographs of wheat samples were obtained by the technic outlined by Milner, Lee, and Katz (2) with a Picker industrial X-ray unit containing a Machlett water-cooled beryllium window tube operated at 15 m.a. and at potentials of from 15 to 25 kilovolts. Exposures were made on 5 x 7 in. sheets of Kodak type A industrial X-ray film processed according to the manufacturer's instructions.

Determinations of specific gravity of wheat samples were made with 25 ml. pycnometer bottles using toluene following essentially the method outlined by Sharp (5).

Tests for hardness were conducted using the Brabender hardness tester, which is essentially a small burr mill fitted to the dynamometer coupling of the Farinograph in place of the regular dough mixing bowl. A description of this apparatus may be found in a recent publication by Paukner (4) who applied it in a study correlating the hardness of malted barley with its content of malt solubles.

[2] The spring wheat samples were supplied through the courtesy of Mr. John MacKenzie of the Department of Mechanical Engineering, University of Minnesota, and Dr. J. A. Anderson, Grain Research Laboratory, Winnipeg, Manitoba, Canada.

Results

Preliminary Study of Factors Causing Internal Fissuring of Vitreous Wheat. As a consequence of the initial discovery of fissures in weathered wheat, a preliminary experiment involving wetting and drying of the grain was carried out. A sample of sound vitreous Pawnee seed wheat of the 1950 Kansas crop containing 11.7% moisture was treated in different ways including wetting to about 20% moisture followed by drying under various conditions. The grain was radiographed to determine fissure formation. The experimental details and the results of this preliminary experiment appear in Table I.

TABLE I

INFLUENCE OF WETTING AND DRYING ON INTERNAL FISSURING OF NON-WEATHERED WHEAT (PRELIMINARY STUDY)

Sample Number	Treatment	Moisture		Radiographic Appearance
		Initial	Final	
		%	%	
1	None	11.7	..	No fissures
2	Wetted, air dried, 25°C., 4 days	20.4	10.4	No fissures
3	Sample 2 rewetted, air dried, 25°C.	19.7	air dry	Some fine fissures
4	Wetted, oven dried 130°C., 30 min.	20.4	9.3	Numerous fissures
5	No wetting, oven dried 130°C., 120 min.	11.7	3.4	No fissures

Sample No. 1, the untreated vitreous seed exhibited no fissures when radiographed. Sample No. 2 which had been wetted to 20.4% moisture and then air-dried at room temperature for 4 days to a moisture content of 10.4% similarly exhibited no internal cracking. However, when this once-wetted and dried sample was rewetted to 19.7% moisture and again air-dried, the resulting sample exhibited internal fissuring. When the tempered sample (20.4% moisture) was dried very rapidly at a high temperature (130°C.) numerous internal fissures appeared (Sample No. 4). Similar drastic drying conditions applied to the non-wetted wheat (Sample No. 5) produced no fissuring.

These preliminary data indicated that radiographically detectable fissuring of a kind similar to that noted in naturally weathered grain

can be reproduced in the laboratory under conditions similar to those occurring in the wheat field. Furthermore it was apparent that the more rapid the drying conditions, the more readily were such fissures formed.

Influence of Wetting to Various Moisture Contents and Drying at Fixed Temperatures on Fissure Formation and Grain Density. To determine the relationship of initial moisture value, temperature and time of drying on the formation of internal fissures, as well as changes in density which occur in grain wetted after drying, the Pawnee seed sample was conditioned to 15, 20, 30, and 40% moisture content and subsamples of these lots were dried at 35°C. and 50°C. for intervals of 1 to 24 hours. The results of the study at the 35°C. drying temperature appear in Table II and those for the 50°C. temperature in Table III. Densities are expressed on the "as is" moisture basis.

TABLE II

EFFECT OF DRYING AT 35°C. ON NON-WEATHERED WHEAT CONDITIONED TO VARIOUS MOISTURE CONTENTS

Moisture		Density	Internal Fissures (from radiograph)
Initial	Final		
%	%		
		No drying	
11.7	(original sample)	1.420	none
14.7	...	1.417	none
19.7	...	1.403	none
30.0	...	1.401	none
40.4	...	1.388	none
		Dried 2 hours	
14.7	12.4	1.400	none
19.7	15.9	1.375	none
30.0	22.7	1.330	none
40.4	31.5	1.290	none
		Dried 5 hours	
14.7	10.3	1.402	none
19.7	12.3	1.387	none
30.0	15.2	1.360	some
40.4	18.4	1.349	numerous
		Dried 24 hours	
14.7	7.4	1.414	none
19.7	7.9	1.411	none
30.0	7.9	1.379	numerous
40.4	8.0	1.384	numerous

The simple addition of water to the grain resulted in a reduction in density in proportion to the moisture content of the sample. On sub-

TABLE III

EFFECT OF DRYING AT 50°C. ON NON-WEATHERED WHEAT CONDITIONED TO VARIOUS MOISTURE CONTENTS

Moisture		Density	Internal Fissures (from radiograph)
Initial	Final		
%	%		
		Dried 1 hour	
15.1	10.0	1.397	none
19.9	15.4	1.383	few
29.8	23.1	1.335	none
40.0	32.9	1.291	none
		Dried 2 hours	
15.1	9.9	1.402	none
19.9	12.4	1.382	few
29.8	17.5	1.354	moderate number
40.0	23.3	1.328	none
		Dried 5 hours	
15.1	8.1	1.414	few
19.9	9.1	1.380	moderate number
29.8	10.5	1.370	many
40.0	11.2	1.362	many
		Dried 10 hours	
15.1	7.5	1.413	moderate number
19.9	7.8	1.394	many
29.8	8.1	1.379	severe
40.0	8.0	1.365	severe

sequent drying the density increased but in no case was the original density of the grain (1.420) regained (Table II). These results indicate the decrease of density occurring with wetting is proportional to the moisture content of the conditioned grain and that the regain in density with drying is in inverse proportion to the initial moisture value, confirming the findings of both Sharp and Swanson.

Under the more severe conditions of drying (50°C.) internal fissures appeared not only sooner than when drying at 35°C. but also the internal cracking occurred in samples with initially lower moisture values (15%). Fissuring in samples of this low moisture value did not occur at the lower drying temperature (35°C.). It is clear that the extent of fissuring increased greatly with the drying temperature and initial moisture content as indicated by the data for the samples conditioned to 30 and 40% moisture. It may be concluded that fissuring during the drying process is favored by elevated initial moisture value and increased drying rate.

Relation of Internal Fissuring to Wheat Hardness. The extent of internal cracking of wheat in naturally weathered grain as well as in

the samples prepared in the laboratory suggested that this condition probably influences the work required to break or crush the grain.

A preliminary test using the Brabender grain hardness tester was carried out with the Pawnee seed wheat sample which had received no treatment as well as with one lot which had been wetted to 30% moisture and dried at 35°C. The hardness curves for these samples as well as a similar set obtained with commercial wheat dried at 130°C. appear in Fig. 1. Radiographs of all samples showed that considerable internal fissuring existed in the dried Pawnee sample (B) and that very severe fissuring appeared in the oven dried commercial sample (H). The untreated samples exhibited no internal cracking. The height of the curves appears to reflect differences in hardness due to extent of internal fissuring and of the treatments accorded these two samples. It is also to be noted that the time necessary for the samples to flow through the grinding elements of the hardness tester was about the same for both the normal and the internally fissured grain. Thus if the areas of these curves may be taken as work functions, not only is the hardness reduced due to internal cracking but also the energy requirement for grinding a sample of given size is decreased.

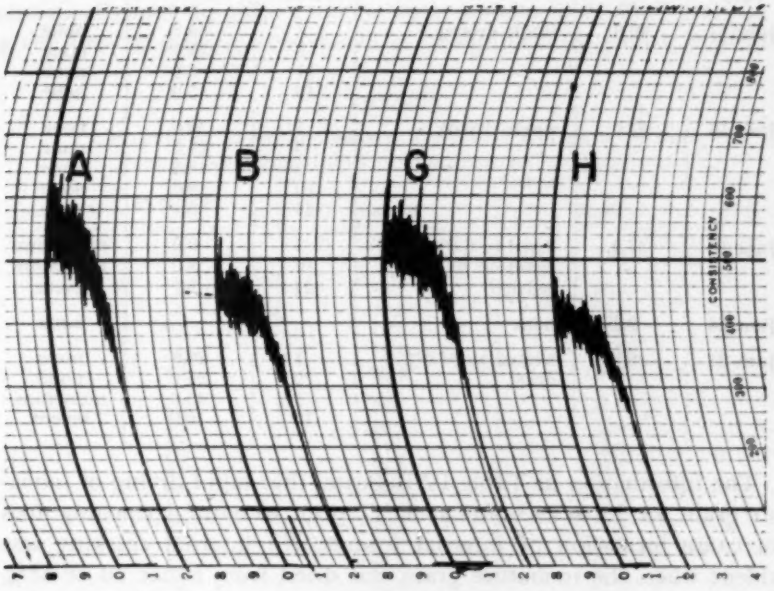

Fig. 1. Hardness curves for non-fissured and fissured wheat. Curve A is for Pawnee seed wheat and curve B is for the same wheat wetted to 30% moisture and dried at 35°C., a treatment which produces fissuring. Curve G is for normal sample of commercial wheat and curve H is for the same grain wetted to 30% moisture and redried to 12% at 130°C.

Relation of Stage of Maturity to Fissure Formation on Drying. The probability that internal fracturing of grain is dependent upon the degree of swelling associated with hydration, made it of interest to determine whether immature wheat in the field, and in the so-called milk and dough stages might be affected also by rapid drying. For this study samples of Pawnee wheat from a plot on the Experiment Station Agronomy Farm at Manhattan were harvested and threshed by hand at two stages before maturity. The first sample was in the late milk stage and the second in the dough stage. These moist samples were dried at 50°C. and then examined radiographically. One of these artificially dried samples then was rewetted to 31.9% moisture and again redried at 50°C. to determine if internal fissuring had occurred. The results of this study appear in Table IV.

TABLE IV

EFFECT OF DRYING IMMATURE WHEAT AT 50°C. ON INTERNAL FISSURING

Date of harvest	Moisture at harvest	Treatment	Drying Time	Moisture After Drying	Internal fissures
1952	%		*hrs.*		
June 11	49.9	single drying	1	45.7	none
			5	19.9	none
			17	6.5	none
			19	5.9	none
June 16	37.9	single drying	1	30.0	none
			3	25.2	none
			5	18.9	none
			10	9.0	none
			24	6.8	none
June 16	37.9	dried to 6.8%	5	9.9	numerous
		rewetted to 31.9%	10	8.2	numerous
		redried	24	7.7	severe
June 24	9.9	none	..	..	none
June 24	9.9	wetted to 31.0%	1	29.0	none
		dried	2	25.2	none
			5	15.8	numerous
			10	10.0	severe

The information in Table IV indicates that rapid drying of immature grain for 10 to 24 hours at 50°C. prior to natural ripening does not cause formation of internal fissures. No internal cracking was evident when the immature grain was dried from either 50 or 38% moisture. On the other hand when the immature sample harvested on June 16 and dried to 6.8%, was rewetted and redried, numerous fis-

sures appeared. The final sample taken when the plot was ripe contained 9.9% moisture. This normal unfissured wheat when rewetted and redried also exhibited considerable internal fissuring.

Influence of Fissuring on the Rate of Water Absorption by Wheat. It appeared probable that the presence of fissures in weathered wheat would influence the rate of moisture uptake during tempering since spaces of capillary dimension were involved. To investigate this matter a sample of the 1952 crop Pawnee wheat containing 10.1% moisture was conditioned to 40% moisture and oven dried for 10 hours, at which time a moisture content of 9.4% was attained and the grain showed extensive fissuring. Twenty-five gram quantities of both the fissured and the normal wheat were placed in small wire sieves and submerged in distilled water for time intervals varying from 5 minutes to 2 hours. At the end of each interval a sample was removed and drained. To further assure the removal of surface water, the grain was transferred to a large stoppered centrifuge tube containing a plug of absorbent glass wool on the bottom, and centrifuged at 2000 r.p.m. for 5 minutes. The samples then were placed in stoppered bottles and duplicate two-stage determinations of moisture content were made. The results are presented in Table V. These data have been analyzed statistically and the differences in water uptake by fissured grain at a given time of soaking were shown to be significantly greater than that

TABLE V

INFLUENCE OF INTERNAL FISSURING ON WATER UPTAKE BY WHEAT

Time of soaking	Moisture Content[1]	
	Normal	Fissured
min.	%	%
5	14.2	16.3
15	17.9	17.2
20	18.3	19.6
30	19.3	19.9
35	20.5	20.3
40	19.7	21.6
45	20.2	22.0
50	21.9	23.6
55	21.2	22.7
60	22.6	23.5
65	22.7	24.5
70	22.2	24.0
80	23.1	25.3
90	22.9	25.7
120	25.9	27.9

[1] Mean for duplicate determinations.

of non-fissured grain. Larger differences in moisture uptake occurred at longer than at shorter times of soaking. These results indicate quite conclusively that internally fissured, naturally weathered wheat would take up more water under the same conditions of wetting than does normal grain, probably due to the capillary effect of the fissures.

Discussion

The detection of internal flaws in solid bodies by radiography is limited by the ratio of the void to the total diameter of the body. The low voltage X-rays used in this investigation apparently permit radiographic visualization of fissures greater than approximately 2% of the kernel diameter. Internal cracks smaller than this ratio would not impart a radiographic shadow. It is apparent therefore that the fissures observed in this study are only those as large or larger than this critical size and that probably many others of smaller dimension exist which were not visible in the radiographs. This consideration suggests that fissuring occurs under very mild conditions of wetting and drying. Thus, for example, Table 1 indicates that no fissures appeared in wheat wetted once to 20.4% moisture and then air dried at room temperature, whereas fissures did appear when this grain was again rewetted and dried under the same mild conditions. It seems highly probable that fine fissures did exist in the sample wetted and dried only once which were not detectable by the X-ray.

The more drastic the conditions of wetting and drying in terms of increased initial moisture content and elevated drying temperature, the more readily were visible fissures produced. The data indicate that there is no critical moisture or temperature at which radiographic fissures appear since the higher the initial moisture content, the higher was the moisture value during the drying process at which fissuring appeared. Similarly, the higher the drying temperature the lower the initial moisture needed to cause cracking. These data suggest that cracking occurs when a certain degree or rate of shrinkage of the hydrated endosperm is exceeded. Thus samples wetted to moderate moisture values (*ca* 15%) did not fissure when dried at temperatures of 35°C. indicating that either the degree of swelling or the rate of drying was insufficient to produce the shrinking required for fissuring. The mechanics of this kind of cracking, due primarily to stresses arising from unequal moisture gradients, have been treated thoroughly by Earle and Ceaglske (1) in a study of factors causing checking in macaroni.

The investigation provides additional evidence that the loss of

water from maturing grain in the field involves more than a simple dehydration process. One factor which may be involved is an increase in chemical aggregation of the endosperm proteins. It may be conceived that the immature grain contains highly hydrated and elastic protein molecules of the fibrous type which are converted during maturation to molecules of the more ramified globular kind, with an accompanying loss in elastic properties. The formation of numerous intramolecular bondings characteristic of globular proteins would confer a greater rigidity to the molecules than do hydrogen bonds and associated oriented water molecules which are replaced. This globular protein which forms with drying may swell on subsequent wetting due to colloidal forces, but its lack of elasticity in the dry state would become manifested by actual breakage of inter- and intra-molecular linkages as the drying proceeds. An analogous structural transition occurs in the case of the carbohydrates as soluble sugars are transformed into insoluble starch granules within the endosperm cells. It seems highly probably that the difference in response of immature grain and wetted mature grain to drying involves structural processes of this kind and that the fissures are evidence of the high rigidity of the endosperm constituents in the dry state.

The decrease in density associated with the weathering of wheat appears to be due simply to the air spaces which prevent a complete reversal of the swelling process which occurs on wetting. Bulk density (test weight) would also be affected due to the roughening and separation of the bran coat with weathering, which counteracts the packing tendency of grain.

Numerous practical inferences flow from the results of the present study. The increased rate of penetration of water into sound grain indicates that weathered wheats would require less tempering time than is necessary with vitreous grain. The decrease in hardness and energy requirement for grinding a weathered sample suggests that weathered wheat is easier to process during milling than is vitreous grain. Several millers have observed this difference. It might be possible to improve the grinding characteristics of vitreous wheat by a process of artificial weathering prior to milling of the kind used to produce fissured wheat in this study which would involve a wetting and drying step in the wheat conditioning process. Certain wheat conditioning processes now in use in the milling industry involve treatment of excessively vitreous grain by steaming and cooling or drying but whether such treatment produces detectable fissuring is not known. From the present study it appears that cracking of this type requires the preliminary addition of 20% or more of moisture,

a condition which is probably not achieved in conditioning methods now in use.

The presence of internal fissures would probably influence the storage character of weathered grain since fungal growth might be enhanced not only due to roughening of the seedcoat but also by the presence of the internal fissures. This possibility also will need additional investigation.

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FURTHER STUDIES OF THE HEAT-LABILE LOAF VOLUME DEPRESSANT OF MILK SERUM PROTEINS[1]

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ABSTRACT

In continuation of previous work on the heat-labile loaf volume depressant of milk serum proteins, several fractions of that mixture were prepared and tested for baking quality. Of the components of the "lactalbumin" (fraction precipitated between 43 and 80% saturation with ammonium sulfate) neither β-lactoglobulin nor an "insoluble protein" isolated from the mother liquor remaining after crystallizing β-lactoglobulin exhibited the depressing effect. Furthermore tests on the entire "lactalbumin" fraction indicated that it does not contain high concentrations of the depressant. Rather, it is concentrated in the classical "lactoglobulin" fraction (proteins precipitated by 43% saturation with ammonium sulfate). Since about 40% of "lactoglobulin" fraction consists of two proteins designated eu- and pseudo-globulin and since these proteins, obtained from colostrum, were previously shown not to depress loaf volume, the depressant is suspected of being present in the remaining 60% of the "lactoglobulin."

The good baking quality of crystalline β-lactoglobulin and other purified milk serum proteins is not due to alterations produced by exposure to high concentrations of ammonium sulfate since the depressing action of the milk serum protein mixture was not eliminated by such treatment.

In a recent paper (4) it was shown that the heat-labile loaf volume depressing agent of unheated milk serum proteins is not associated with four individual proteins which have been isolated heretofore from that mixture (i. e. β-lactoglobulin, serum albumin, euglobulin, and pseudoglobulin). Furthermore, since β-lactoglobulin is the principal sulfhydryl-containing protein of the mixture, the loaf volume depression does not appear to be due to the reducing action of sulfhydryl groups on gluten as was formerly thought to be the case (3, 6).

These rather surprising results may be explained in two ways. Either the factor which is sought resides in one or more of the four proteins previously investigated but is altered or destroyed by the isolation processes employed (particularly treatment with high concentrations of ammonium sulfate) or it resides in some other component than these four. The work reported in the present paper was undertaken in an attempt to answer these questions.

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Classically the milk serum protein mixture was often fractionated at a pH near neutrality with ammonium sulfate into "lactoglobulin" and "lactalbumin," the former being precipated by approximately 50% saturation and the latter between 50% and 80% saturation. Each of these fractions is a mixture and has been resolved into several components. Thus Smith (5) isolated from "lactoglobulin" by further fractionation with ammonium sulfate two relatively homogeneous proteins, eu- and pseudo-globulin, which together comprise about 40% of the "lactoglobulin." The remaining 60% consists of components not isolated as yet. The principal component of "lactalbumin" is the well-known β-lactoglobulin which crystallizes upon exhaustive dialysis of "lactalbumin." The mother liquor remaining after crystallization of β-lactoglobulin contains a number of protein components. One of these, representing about 5% of the entire serum proteins, is identical to bovine blood serum albumin. Another was first crystallized by Sorenson and Sorenson (7) and described as an "insoluble protein" because it is less soluble than β-lactoglobulin in dilute salt solution at pH 4.6.

In the work reported herein the "insoluble protein" was isolated in a relatively pure state for baking studies. In further experiments the effects of treatment with high concentrations of ammonium sulfate on the baking behavior of milk serum proteins were determined and the baking qualities of the classical "lactalbumin" and "lactoglobulin" fractions were investigated.

General Procedures

All protein preparations were made from skimmilk obtained from the University dairy. The milk serum protein mixture was prepared by precipitation of the casein with acetate buffer, dialysis of the filtrate, and freeze-drying as described previously (4).

In fractionating the serum protein mixture into "lactoglobulin" and "lactalbumin," the former was precipitated at 43% saturation, pH 6.5, with ammonium sulfate[4], and the latter by bringing the ammonium sulfate concentration of the first filtrate to 80%. The choice of 43% saturation for this separation was based on unpublished experiments which showed that an optimum yield of β-lactoglobulin can be obtained from lactalbumin precipitated between 43 and 80% saturation.

The baking test was conducted in the same manner as that employed in a previous study (4). All protein preparations were incor-

[4] The term "percentage saturation" as employed throughout this paper refers to the extent of saturation of water in the preparation. It was computed on the basis of a solubility of 770 g. of ammonium sulfate per 1000 g. of water at 25°C. (8).

porated into the dough in the form of sols in phosphate buffer (pH 6.9, $\mu = 0.1$). In every case 40 ml. of sol, containing an amount of protein approximately equivalent to the amount of that fraction in 0.5 g. of serum protein mixture (or in 6 g. of nonfat dry milk solids), was used per 100 g. of flour.[5] The amounts of protein employed were calculated from the yields of fractions actually obtained. The bromate level was 1 mg. per 100 g. of flour in all cases.

Isolation and Baking Behavior of the "Insoluble Protein"

The "insoluble protein" was isolated from the mother liquor remaining after β-lactoglobulin had been crystallized (according to the procedure of Sorenson and Sorenson (7)). This mother liquor was adjusted to pH 4.0 and saturated to the extent of 35% with ammonium sulfate. The resulting precipitate was filtered off, and washed thoroughly with a 35% saturated ammonium sulfate solution. It was then dissolved in water and dialyzed free of ammonium sulfate whereupon the insoluble protein precipitated and was filtered off. Electrophoretically this material in veronal buffer at pH 8.7, $\mu = 0.1$ yielded a monophoretic pattern and a mobility of -3.9×10^{-5}cm.^{2}/volt/sec. At pH 6.9, $\mu = 0.1$, in phosphate buffer small amounts of contaminants of higher mobility were noted. The material was not crystallized.[6]

The baking behavior of the "insoluble protein" is shown in Table I, unheated serum protein being included for comparison. Evidently this protein is not the loaf volume depressant.

TABLE I

BAKING BEHAVIOR OF "INSOLUBLE PROTEIN" AND SERUM PROTEIN MIXTURE

Sample	Amount added per 100 g. flour	No. of Replicates	Mean Loaf Volume[1]
	mg.		*cc.*
Buffer control	...	4	798
Serum protein	500	4	689
"Insoluble protein"	100	3	770

[1] Standard error of single determination in this series was 12.2 cc.

[5] Some of the filtrates were concentrated to a certain degree (as noted in the tables) and equilibrated against phosphate buffer. Forty ml. of the concentrate were employed per 100 g. of flour.

[6] During the course of this work a brief note on the crystallization of this protein was published by Gordon and Semmett (2) and prior to publication the details of their method were published (J. Am. Chem. Soc. 75: 328, 1953).

Effect of Ammonium Sulfate on Baking Behavior of Milk Serum Protein

The next experiment was performed to determine if exposure of the milk serum protein mixture to high concentrations of ammonium sulfate without fractionating inactivates the deleterious baking factor. Nine grams of freeze-dried serum protein were dispersed in 760 ml. of phosphate buffer, (pH = 6.4, $\mu = 0.1$). To 540 ml. of this sol at 25°C. was added 330 g. of ammonium sulfate (80% saturation of the water). The preparation was held for 3.5 hours, the precipitated protein suspended by mixing and the mixture dialyzed at approximately 5°C. in Visking casings against distilled water until sulfate could no longer be detected in the dialysate (about 7 days were required). The remaining 220 ml. of the protein solution was not treated with ammonium sulfate but was dialyzed against distilled water for an equal period of time. Finally both sols were equilibrated against phosphate buffer, pH 6.9, $\mu = 0.1$ and used for the baking test. The baking results, shown in Table II, indicate that the exposure to high concentrations of amonium sulfate does not materially influence the deleterious baking effect of milk serum proteins.

TABLE II

EFFECT OF TREATMENT WITH AMMONIUM SULFATE ON THE BAKING BEHAVIOR OF MILK SERUM PROTEINS

Sample	Amount added per 100 g. flour	No. of Replicates	Mean Loaf Volume[1]
	mg.		*cc.*
Buffer control	...	6	801
Serum protein	500	4	676
Serum protein treated with 80% saturated $(NH_4)_2SO_4$	500	4	689

[1] Standard error of single determination in this series was 17.0 cc.

Baking Behavior of Fractions Obtained in Isolation of β-Lactoglobulin

An experiment was next devised to determine if the process of preparing crystalline β-lactoglobulin is responsible for its excellent baking behavior. This involved a comparison of the baking quality of serum protein, "lactalbumin," crystalline β-lactoglobulin, and mother liquor from crystallization of β-lactoglobulin. These protein frac-

tions were all prepared from a single lot of 11 l. of fresh skimmilk. Serum protein was prepared from a 2 l. portion by acetate buffer precipation of the casein, dialysis, and freeze-drying. The remaining 9 l. was 43% saturated with ammonium sulfate and after 4 hours the precipitate of casein and lactoglobulin was filtered off and discarded. The filtrate, containing the lactalbumin, was dialyzed against distilled water for 4 days and then divided into two portions. One-third of it was dialyzed free of ammonium sulfate and freeze-dried while the remaining two-thirds was pervaporated to one-third of its volume, and, after adjusting the pH to 5.17 with normal sulfuric acid, it was further dialyzed and finally seeded with β-lactoglobulin crystals. The crop of β-lactoglobulin obtained was recrystallized three times by dissolving in 0.1 *N* sodium chloride and dialyzing. All mother liquors were combined, dialyzed, and freeze-dried. The results, given in Table III, show that the good baking quality of β-lactoglobulin does not arise as a result of the processes of isolation.

TABLE III

COMPARATIVE BAKING PROPERTIES OF SERUM PROTEIN AND SEVERAL LACTALBUMIN FRACTIONS[1]

Sample	Amount added per 100 g. flour	No. of Replicates	Mean Loaf Volume[2]
	mg.		*cc.*
Buffer control	...	2	788
Serum protein	500	4	656
Lactalbumin	450	4	746
Crystalline β-lactoglobulin	250	4	739
Mother liquor from β-lactoglobulin	200	4	747
β-lactoglobulin plus mother liquor	450	4	729

[1] All preparations made from a single lot of fresh skimmilk (Lot A).
[2] Standard error of single determination in this series was 14.8 cc.

Comparative Baking Quality of "Lactalbumin" and "Lactoglobulin"

Since, in the preceding experiment, the "lactalbumin" fraction was of relatively good baking quality, attention was immediately focused on the "lactoglobulin" fraction as a possible source of the deleterious baking factor. To obtain samples for a comparative baking test, the casein was acid precipitated from a lot of fresh skimmilk. The acid

whey was neutralized with normal sodium hydroxide solution to pH 6.5 after which "lactoglobulin" was precipitated at 43% saturation and "lactalbumin" between 43 and 80% saturation with ammonium sulfate. These two precipitates and the filtrate obtained at 80% saturation were separately dialyzed, equilibrated against phosphate buffer

TABLE IV

BAKING BEHAVIOR OF WHEY FRACTIONS INDIVIDUALLY AND IN COMBINATION[1]

Sample	Amount added per 100 g. flour	No. of Replicates	Mean Loaf Volume[2]
	mg.		*cc.*
1. Buffer control	...	2	793
2. Serum protein	500	4	689
3. Lactalbumin	375	2	738
4. Lactoglobulin	125	4	674
5. Filtrate from 80% saturation of whey with $(NH_4)_2SO_4$ (conc. 3:2 and dialyzed vs. buffer)	...	2	798
3 + 4 + 5	500	2	675

[1] All preparations made from a single lot of fresh skimmilk (Lot B).
[2] Standard error of single determination in this series was 11.2 cc.

TABLE V

EFFECT OF HEAT TREATMENT ON BAKING BEHAVIOR OF SERUM PROTEIN, LACTALBUMIN, AND LACTOGLOBULIN

Sample	Amount added per 100 g. flour	No. of Replicates	Mean Loaf Volume[1]
	mg.		*cc.*
Series from Skim Lot B:			
Buffer control	...	6	807
Serum protein	500	4	688
Serum protein heated[2]	500	2	788
Lactalbumin	375	2	738
Lactalbumin heated[2]	375	2	758
Lactoglobulin	125	4	686
Lactoglobulin heated[2]	125	4	799
Series from Skim Lot C:			
Buffer control	...	3	813
Lactoglobulin	125	2	685
Lactoglobulin heated[2]	125	2	788

[1] Standard error of single determination in this series was 10.3 cc.
[2] Heated at 80°C. for 30 min. in phosphate buffer solution.

and baked. As shown in Table IV the deleterious baking factor is for the most part present in the "lactoglobulin" fraction. This finding was corroborated in another series of baking tests on the same group of samples and the results are reported in Table V. In this latter series it was also demonstrated that "lactoglobulin" responds to heat treatment in the same way as does the entire serum protein mixture.

Baking Behavior of "Lactoglobulin" Fractions

It was next sought further to concentrate the heat-labile deleterious factor by fractionation of the "lactoglobulin." The procedure proposed by Smith (5) for fractionating "lactoglobulin" into two portions containing respectively 20 and 80% of the immune globulins appeared promising, particularly in view of the fact that eu- and pseudo-globulins from colostrum had previously been shown to be of good baking quality (4). The fraction containing the smaller quantity of immune proteins might be expected to contain more of the loaf volume depressant. From a lot of "lactoglobulin," successive precipitates were obtained at pH 4.6, and 25% saturation with ammonium sulfate and pH 6.0 and 40% saturation. These two precipitates and the filtrate from the latter were dialyzed and equilibrated against buffer for baking. The results, shown in Table VI, indicate that the deleterious agent is divided about equally between the two precipitates although the second is much richer in immune globulins.[7]

Discussion

The findings reported herein corroborate those of Larson, *et al.* (4) in showing that β-lactoglobulin does not contain the deleterious baking factor of milk serum proteins. A fifth protein, the "insoluble protein" of Sorenson and Sorenson may now be added to the list of those that do not contain the factor. The results further demonstrate that the good baking qualities of β-lactoglobulin and the other purified proteins are not artifacts resulting from the treatments employed in isolating them.

The possibility that the loaf volume depression produced by unheated milk serum proteins involves an action of sulfhydryl groups appears to be rather remote. β-lactoglobulin, the principal sulfhydryl containing protein of the mixture does not exhibit the effect and the "lactoglobulin" fraction which does exhibit it has a very low sulfhy-

[7] Verified by electrophoretic analysis.

dryl content.[8] Of course, it cannot be entirely precluded that a protein fraction present in very low concentration and containing very active sulfhydryls is the agent in question.

TABLE VI

BAKING BEHAVIOR OF LACTOGLOBULIN FRACTIONS[2]

Sample	Amount added per 100 g. flour	No. of Replicates	Mean Loaf Volume[1]
			cc.
1. Phosphate buffer (pH 6.9, $\mu = 0.1$)	40 ml.	4	805
2. Lactoglobulin	125 mg.	2	693
3. Filtrate from reprecipitation of lactoglobulin at 43% saturation with $(NH_4)_2SO_4$ (concentrated 3:2 and dialyzed vs. buffer)	40 ml.	2	780
4. Precipitate, pH 6.0 25% saturation	75 mg.	2	728
5. Precipitate[3], pH 6.0 40% saturation	50 mg.	1	735
6. Filtrate, pH 6.0 40% saturation (concentrated 3:2 and dialyzed vs. buffer	40 ml.	2	782
4 + 5 + 6	125 mg.	2	698

[1] Standard error of single determination in this series was 14.2 cc.
[2] All preparations from a single lot of fresh skimmilk (Lot B).
[3] This precipation made subsequent to removal of precipate at pH 4.6, 25% saturation.

Throughout this paper the fraction of milk serum proteins precipitated by 43% saturation with ammonium sulfate has been designated "lactoglobulin" whereas that not so precipitated is called "lactalbumin." Obviously this distinction is largely one of convenience and does not imply a sharp separation of chemical entities. Each fraction contains a number of proteins and certain components doubtless are partitioned between the two. Indeed the data indicate some partition of the deleterious baking agent since "lactalbumin" always depressed loaf volumes to some extent (43, 55, and 66 cc. in Tables III, IV, and V respectively). However, there is no question as to the presence of the overwhelming majority of the agent in the "lactoglobulin" fraction as evidenced by depressions of 119, 128, 121, and 112 cc. in

[8] Titration by the method of Larson, et al. (4) showed "lactoglobulin" and "lactalbumin" from a single lot of skimmilk to contain respectively 0.011 and 0.080 m. eq. of sulfhydryl per gram.

Tables IV, V, and VI respectively. This large depression is produced by only 125 mg. of protein per 100 g. of flour and the active agent may indeed represent only a small fraction of that amount. Thus, it is evidently a material capable of exerting a powerful action, but the mechanism of this action is unknown at present.

Some possibilities that might be suggested as to the nature of the active agent are: (1) that it is a protease capable of attacking gluten, (2) that it is an activator of latent proteases of flour, (3) that it is an amylase which attacks starch, or (4) that it is an activator of yeast protease or an inhibitor of yeast fermentation.[9] Some of these possibilities will be considered in a subsequent paper (1).

The fact that the deleterious baking agent resides in the "lactoglobulin" fraction whereas eu- and pseudo-globulin, which comprise about 40% of that fraction, apparently do not contain it (4) would indicate that the agent should be sought among the other components of "lactoglobulin." This possibility should be investigated, but at the same time it should not be forgotten that the eu- and pseudo-globulin preparations employed in the earlier baking studies were isolated from colostrum, rather than from normal milk. Possibly eu- and pseudo-globulin preparations from milk would behave differently in baking than those from colostrum.

Acknowledgment

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[9] Admittedly the fact that unheated milk serum protein exerts a dough softening effect in the absence of yeast makes this last possibility rather doubtful.

THE DISTRIBUTION OF THIAMINE AND RIBOFLAVIN IN THE MUNG BEAN; AND THE CHANGES THAT OCCUR DURING GERMINATION[1]

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ABSTRACT

The distribution of thiamine and riboflavin in the dormant seeds of the Mung bean (*Phaseolus aureus*) has been studied photographically, together with the changes that occur in the distribution of these vitamins in the seed during the early stages of germination.

Thiamine appears, in the dormant seed, to be concentrated mainly in the cells of the embryo and of the cotyledons adjacent to the embryo, whereas some concentration of riboflavin appears to occur in the cells of the radicle, with a lesser amount in the cotyledons. General diffusion of both vitamins throughout the tissues of the cotyledons appears to take place at the commencement of germination, possibly as a result of translocation by the absorbed water.

The presence of cells, apparently rich in riboflavin and to a lesser extent in thiamine, has been shown dispersed throughout the tissues of the cotyledons in the dormant seed. It is possible that these cells are those of vascular bundles.

Sprouted pulses are common articles of food in Malaya and other countries in South East Asia. The commonest pulse used for this purpose in Malaya is probably the Mung bean or Green Gram (*Phaseolus aureus*). The sprouted beans are produced on a large scale commercially and are known as *Towgay* or *Tau Geh*. The usual method employed for germination consists of enclosing the beans in wicker baskets which are kept moist with well water for three or four days, by which time the young shoots have attained a length of two or more inches. The beans are not kept in the dark during germination although they are not normally exposed to direct sunlight. In view of their widespread use as a vegetable, it was of interest to examine the changes that may occur in the distribution of thiamine and riboflavin in the bean during germination.

A good summarized description of the behavior of the B vitamins in seeds during germination is given by Bonner and Bonner (2). Reference is made in this article to the demonstration by Bonner and Greene (3) that thiamine is not synthesized to an appreciable degree during the germination of pea seeds in the dark, whereas when the plants are grown in the light, major increases in thiamine occur.

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Similar findings on the effect of light on thiamine synthesis during the germination of seeds are reported by Bonner (1) in the case of tomatoes and by Hoffer and colleagues (9) in the case of wheat. During the initial stages of germination of black-eyed peas and lima beans, Cheldelin and Lane (6) have shown that even when grown in the light, the seedling plant appears to be totally dependent on its reserves of thiamine but is capable of synthesizing riboflavin under these conditions. Burkholder and McVeigh (4) have shown that riboflavin synthesis occurs in the germinating seeds of wheat, corn, and beans, even in the dark. These authors have also reported (5) the behavior of B vitamins during the germination for four days of Soy and Mung beans in the dark. Their results, which are reproduced in Table I, together with some figures recorded by other authors, show that in the Mung bean little change appears to occur in the thiamine content, whereas a considerable increase of riboflavin takes place.

It thus appears that, generally speaking, seeds rely largely on the stored reserve of thiamine during germination when this occurs in the dark, but that synthesis of thiamine may occur in some species when germinated in the light. In contrast, synthesis of riboflavin apparently can take place during germination in the dark.

TABLE I

THIAMINE AND RIBOFLAVIN CONTENT OF MUNG BEANS (PHASEOLUS AUREUS) AS RECORDED BY VARIOUS AUTHORS

(Results are given as μg./g. of bean on a dry basis)

Authors	Ref.	Thiamine content	Riboflavin content	Remarks
Nicholls	(10)	5.3	3.9	Dormant seed
Guterriez	(7)	10-11	..	Dormant seed
Burkholder and McVeigh	(5)	8.2	3.0	Dormant seed
Burkholder and McVeigh	..	8.4	14.4	Germinated in dark for four days

Hoffer, Alcock, and Geddes (9) have shown that when wheat seeds are germinated in the dark, thiamine accumulates in the embryo at the expense of the endosperm, although the total thiamine in the seed remains constant.

The present work was undertaken to study the changes that may occur in the total amount of thiamine and riboflavin and in their distribution in the seed of the Mung bean when it is germinated under

conditions resembling as closely as possible those obtained when the seeds are germinated on a commercial scale. For this purpose, the photographic technique described by Somers, Coolidge and Hamner (13) and Simpson (12) was used.

Experimental

Samples of Mung beans were purchased on the local market and portions of each sample were germinated in the laboratory at room temperature (about 28°C.) for periods up to 48 hours. The beans were examined before germination and at the following stages during germination:

(a) After about eight hours, mainly in the dark by which time the beans were just beginning to sprout.
(b) After 20 hours, of which about 12 hours were in the dark, when the young shoots had attained a length of about one inch.
(c) After 48 hours, of which about half the time was in the dark. By this time, the young shoots were about two inches in length.

For germination, the beans were spread in a thin layer on moist cloth in a dish placed on a bench in the laboratory. At no time were the beans exposed to direct sunlight.

The original dormant beans and a representative sample at each of the above stages of germination were analyzed for moisture, thiamine, and riboflavin. These vitamins were estimated by methods based on those currently used in our laboratory for cereals (8, 11). An appropriate quantity of beans, after maceration in a Waring blender, was extracted with 0.1 *N* sulphuric acid, the extract incubated with clarase, made up to volume, filtered, and aliquots of the filtrate taken for thiamine and riboflavin analysis. In the case of the former, an aliquot was adsorbed on decalso and the thiamine eluted with 25% potassium chloride in 0.1 *N* hydrochloric acid solution. The thiamine in the eluate was oxidized with alkaline ferricyanide to thiochrome which was extracted with iso-butanol and estimated using a Coleman photoelectric fluorimeter. Blank estimations and recovery estimations of added known amounts of thiamine were also made.

Riboflavin was determined in another aliquot of the original extract, some impurities being removed by oxidation with potassium permanganate, the excess permanganate being discharged with hydrogen peroxide; further impurities were then removed by reduction with

sodium hydrosulphite and stannous chloride and subsequent oxidation of the riboflavin by vigorous shaking in air. The fluorescence was then measured using a Coleman fluorimeter. Allowance was made for non-riboflavin fluorescent impurities by measuring the fluorescence which remained after reduction of the riboflavin with sodium hydrosulphite.

Analyses were made on three different samples of beans which had been purchased at different times. The results recorded in Table II represent the mean of the three sets of results obtained for the dormant beans and for the beans at the three stages of germination already described.

The photographic methods used were similar to those described by Simpson (12). On this occasion, however, it was possible, when checking the selectivity of the light filters with test paper strips, to include a strip which had been moistened with a solution of co-carboxylase; and the observation of Somers and his colleagues (13) is confirmed that the method will not distinguish between co-carboxylase and thiamine (Fig. 1). No distinction can therefore be made in this work between free and phosphorylated thiamine.

TABLE II

THIAMINE AND RIBOFLAVIN LEVELS IN THE MUNG BEAN AT DIFFERENT STAGES OF GERMINATION

(Results are given as μg./g. of bean on a dry basis)

Sample	Thiamine	Riboflavin
Beans, dormant	9.2	4.2
Beans, just sprouted	8.4	2.8
Beans, sprouted about one inch	9.6	3.8
Beans, sprouted about two inches	9.7	5.6

For the photographs, the seeds were cut by hand with a razor to expose, as far as possible, the broadest surface of the section required. In each case, the seeds were sectioned in three planes, namely:

(a) Lengthwise along the hilum—broad longitudinal section.
(b) Lengthwise at right angles to the hilum—narrow longitudinal section.

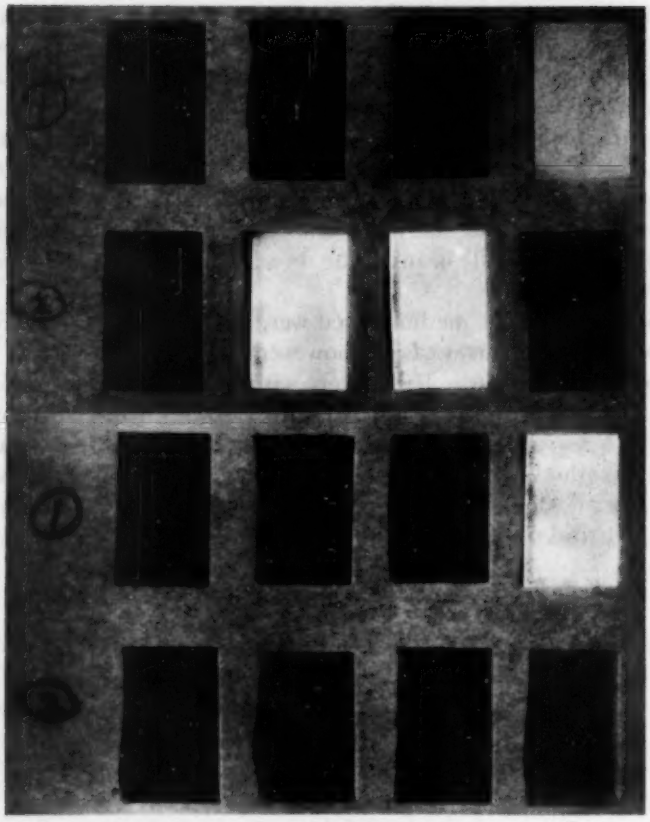

Fig. 1. Photographs of treated paper slips. **TOP** (Group A). Obtained by using filters selective for thiochrome fluorescence, i.e., Wratten filter No. 18A in front of the ultra-violet lamp and Wratten filters Nos. 8 and 39 on the camera. **Top row**, left to right: control; thiamine; co-carboxylase; riboflavin. **Bottom row**, same as top row, except that all strips have been treated with an alkaline ferricyanide solution. **BOTTOM** (Group B). Obtained by using filters selective for riboflavin fluorescence, i.e., Wratten filter No. 36 in front of the ultra-violet lamp and Wratten filters Nos. 2A and 21 on the camera. **Top row**, left to right: control; thiamine; co-carboxylase; riboflavin. **Bottom row**, same as top row, except that all strips have been treated with an alkaline ferricyanide solution.

(c) Across the center of the seed at right angles to the hilum—cross section, center of seed.

The seeds were then mounted, with the cut surface uppermost, on a thin layer of plasticine spread on a small board.

For the photographs intended to show the location of thiamine, (Fig. 2), the exposed cut surface was moistened with a mixture of 5 parts of 5% sodium hydroxide and 1 part of 0.5% potassium ferricyanide by means of a camel's hair brush. In the case of the moist sprouted seeds, it was found necessary to remove with care any surplus

moisture from the cut surface with filter paper and then to place the sections in a desiccator for about 3-4 hours to dry off slightly. The alkaline ferricyanide solution was then applied and the sections again slightly dried in a desiccator for a further 3-4 hours, after which they were photographed. Care was necessary as not to dry out the sections too much since splitting and distortion were liable to occur.

For the photographs designed to show the location of riboflavin (Fig. 3), no treatment of the cut surface of the seeds was required, other than the careful removal of any surplus moisture in the case of the germinated seeds.

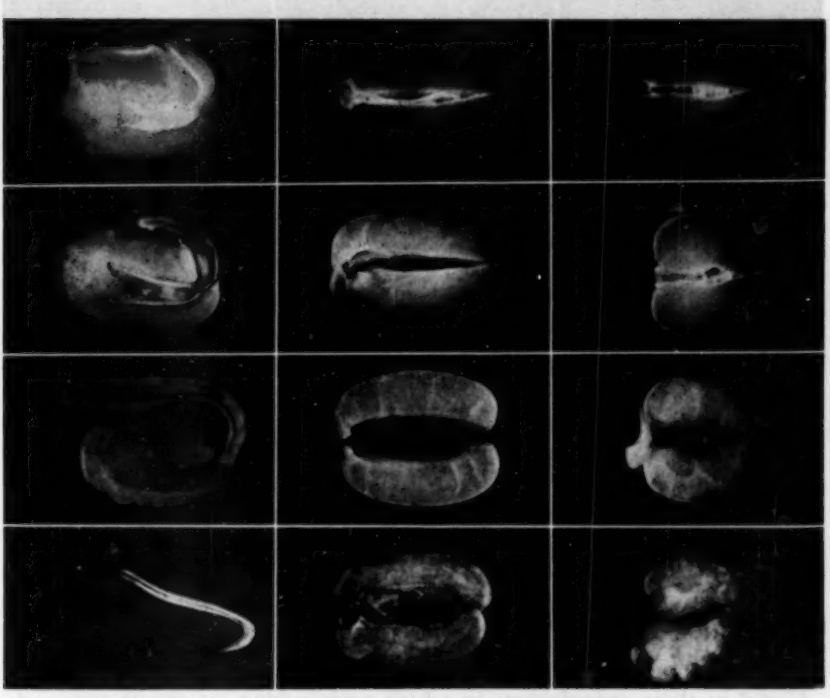

Fig. 2. Location of thiamine in the Mung bean (Phaseolus aureus). Magnification approximately 5X unless otherwise noted. **Top row,** Dormant seed. Left to right: (Same for all rows) broad longitudinal section; narrow longitudinal section; cross section, center of seed. **Second row,** Seed, just commencing to sprout (8 hours). **Third row,** Seed, with shoot about 1 inch long (20 hours). **Bottom row,** Seed, with shoot about 2 inches long (48 hours). Magnification of broad longitudinal section approximately 1½X.

Discussion

The results now reported appear to indicate that, under the conditions of germination described above, the content of thiamine and of riboflavin in the whole seed decreases during the initial stages of

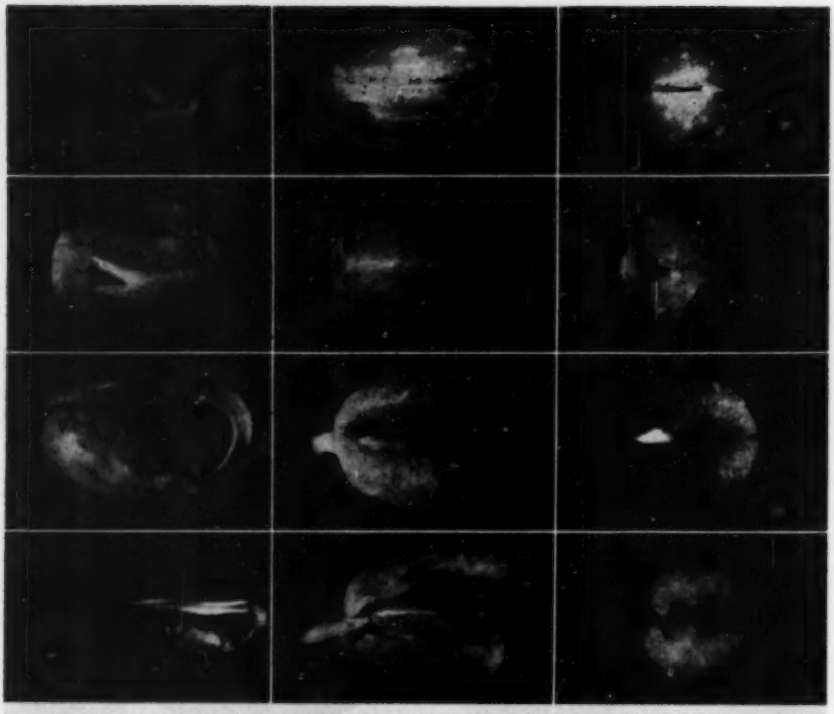

Fig. 3. Location of riboflavin in the Mung bean (Phaseolus aureus). Magnification approximately 5X unless otherwise noted. **Top row,** Dormant seed. Left to right: (Same for all rows) broad longitudinal section; narrow longitudinal section; cross section, center of seed. **Second row,** Seed just commencing to sprout (8 hours). **Third row,** Seed, with shoot about 1 inch long (20 hours). **Bottom row,** Seed, with shoot about 2 inches long (48 hours). Magnification of broad longitudinal section approximately 1½X.

germination. About 20 hours after the commencement of germination the levels of both vitamins have increased; this increase is more pronounced after 48 hours, when the level of thiamine is slightly higher and that for riboflavin appreciably higher than the levels in the dormant bean. The very marked increase in riboflavin after a 4 day germination, as recorded by Burkholder and McVeigh (5), has not been found in these experiments in which germination was not continued beyond 2 days. The results, however, appear to confirm the synthesis of riboflavin in the germinating Mung bean after an initial period during which stored vitamin is utilized. Synthesis of thiamine may also occur but to a much lesser extent.

The photographic records appear to show that in the case of thiamine, the vitamin in the dormant seed is concentrated largely in the cells of the germ and in the cells of the cotyledons adjacent to the germ; and that during the process of germination, more general diffu-

sion throughout the tissues of the cotyledons appears to occur. This may be due to translocation caused by absorption and diffusion of water into these tissues. A marked concentration of the thiamine also appears to develop in cells of the wall of the young growing shoot.

In the case of riboflavin, a more general dispersion of the vitamin appears to occur in the cotyledons of the dormant seed, although the cells of the radicle appear to be richer in the vitamin than the remainder of the seed. As in the case of thiamine, riboflavin also appears to develop in the cells of the wall of the young growing shoot.

An interesting feature, clearly shown in the case of riboflavin, but not quite so obvious in the case of thiamine is the existence of cells, dispersed in the tissues of the cotyledons, which appear to be richer in riboflavin and, to some extent, thiamine than the remainder of the cells of this tissue. It is possible that these cells are those of vascular bundles.

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FACTORS AFFECTING SOLUBILIZATION OF THE NITROGENOUS CONSTITUENTS OF THE MUNG BEAN, PHASEOLUS AUREAS[1,2]

STEPHEN S. T. DJANG, H. A. LILLEVIK, AND CHARLES D. BALL

ABSTRACT

The degree of solubilization of nitrogenous compounds from Mung beans, *Phaseolus aureas*, with 0.4 *M* sodium chloride solutions was enhanced by a decrease in particle size and by employing a high ratio of sample to solvent. With 60 mesh meal, solubilization was essentially complete using either hand or mechanical shaking. By successive extractions, 60% of the nitrogen was removed in the first extract, about 10% in the second and about 5% in a third extraction. The yield of solubilized nitrogen from lipide-free meal was greater than with lipide-containing meal providing the temperature was below about 45°C., whereas at higher temperatures the opposite effect was apparent. The influence of hydrogen ion activity on solubility was reduced by the presence of sodium chloride.

The extraction of proteins from seeds has been reported by many investigators (6-11). In the majority of cases most complete extraction has been accomplished by the employment of acids or bases, the use of the latter usually having been more efficient. In view of the fact that the procedure for extraction should preferably be kept as near to the natural conditions existing in the plant as possible (2), some of the products obtained by acid and base extraction may be considered as artifacts. The conditions laid down for the extraction of proteins in the least altered condition would demand the use of neutral salts (2, 5). The work reported in this paper has considered in detail various factors which would influence neutral salt solubilization of the proteins of the Mung bean, *Phaseolus aureas.*

In previous work on Mung beans (4) salt concentrations from 1 to 0.0001 *M* composed of chloride, sulfate, phosphate, carbonate, and sulfites of sodium and potassium were used as solvents or dispersion media. The range of salt concentrations (as ionic strength) found for maximum solubilization was quite narrow when using solutions of sodium chloride, potassium chloride, sodium sulfate, and potassium sulfate; whereas no such narrow range appeared for carbonates, phosphates, and sulfites of the same cations. The optimal salt concentra-

[1] Manuscript received August 11, 1952. Contribution from the Kedzie Chemical Laboratory, Michigan State College, East Lansing, Michigan.

[2] Abstracted in part from a thesis submitted by Stephen S. T. Djang in partial fulfillment of the requirements for the Ph.D. degree in Biochemistry.

tion for maximum solubilization was found to be about 0.4 *M* for the chlorides and about 0.1 *M* for the sulfates of sodium and potassium. Hence, 0.4 *M* sodium chloride was used as as dispersing agent throughout this work and the amounts of assumed protein solubilized were reported as percentage of total nitrogen in the bean, on an air-dry weight basis.

Materials and Methods

Mung beans[3] were ground in a semi-micro Wiley mill to pass 20, 40, or 60 mesh sieves. The meal was thoroughly mixed. Its proximate analysis gave the following results: Moisture, 8.2%; Ash, 3.1%; Protein (N x 6.25), 23.7%; Lipide, 0.8%; Crude Fiber, 4.4%.

The solubilization studies considered such factors as: (1) ratio of sample weight to solvent volume; (2) extraction time; (3) particle size; (4) successive extractions; (5) mode of stirring; (6) influence of lipides; (7) temperature; and (8) hydrogen ion activity.

Weighed quantities of the Mung bean meal were transferred to 250 ml. centrifuge bottles, which contained a fixed volume of the solubilizing medium. In all cases 0.4 *M* sodium chloride was used except when a comparison was desired with another extracting medium. The bottles, each containing 12 glass beads introduced as agitation aids were placed in a mechanical shaker[4], and subjected to 120 oscillations per minute for a definite time and temperature. The bottles were then centrifuged for 15 minutes at 2000 r.p.m. and the total nitrogen of the supernatant layer was determined by the Kjeldahl-Gunning Method using boric acid as a receiver for the ammonia.

Sample weights varied from 5 to 15 g. and the dispersing solution was kept at 100 ml. Extraction times varied from 10 to 40 minutes. Particle sizes of 20, 40, and 60 mesh were used. Three successive extractions were made upon the same sample and the separate extracts were analyzed for nitrogen. Hand stirring with a glass rod was carried out at occasional intervals and compared with mechanical shaking. Lipide-free meal, extracted by ethanol-ether (3) was compared with the untreated meal, using concentrations of sodium chloride of 0.05, 0.1, and 0.4 *M*. Three temperatures, 25, 45, and 55°C. were employed. Hydrogen ion activity was studied over a range of pH from 1 to 11, the media for solubilization having been prepared by adding 2.34 g. of sodium chloride per 100 ml. at the desired pH. The pH of the extract reported was determined with a Beckman pH meter and

[3] Supplied by the courtesy of the Johnston Seed Company, Enid, Oklahoma.
[4] Cenco-Meinzer Laboratory shaker.

the centrifuged, supernatant material was taken for the nitrogen determination.

Results and Discussion

Examination of Tables I and II shows that the smallest sample to solvent ratio produced the greatest yield of solubilized nitrogen, where-

TABLE I

THE INFLUENCE OF SAMPLE TO SOLVENT RATIO, EXTRACTION TIME, AND PARTICLE SIZE UPON THE SOLUBILIZATION AT 25°C. OF NITROGENOUS CONSTITUENTS OF MUNG BEAN

Amt. Sample per 100 ml. Solvent[1]	Extraction Time	Amount of Total Nitrogen Solubilized According to Particle Sizes		
		20 mesh	40 mesh	60 mesh
g.	*min.*	%	%	%
5.0	10	...	...	67.3
	20	25.7	44.8	69.7
	30	26.3	45.2	70.2
	40	30.3	46.5	70.3
7.5	10	...	...	64.8
	20	24.8	44.1	67.6
	30	25.3	44.4	68.6
	40	29.4	46.1	69.3
10.0	10	...	...	61.3
	20	23.5	43.3	65.0
	30	24.5	43.7	66.6
	40	28.5	45.6	68.1
12.5	10	...	...	57.2
	20	22.2	42.1	57.8
	30	23.4	42.8	64.6
	40	27.8	44.8	66.8
15.0	10	...	...	51.2
	20	20.7	40.7	57.8
	30	22.0	41.5	63.2
	40	26.1	44.1	65.2
10.0[2]	10	...	...	30.3
	20	15.3	27.1	31.8
	30	17.8	29.1	32.4
	40	24.6	30.3	32.7

[1] The solubilizing medium consisted of 0.4 M sodium chloride.

[2] The solubilizing medium consisted of distilled water included for the purpose of comparison.

TABLE II

THE EFFECT OF SAMPLE TO SOLVENT RATIO, NUMBER OF SUCCESSIVE EXTRACTIONS, AND HAND OR MECHANICAL STIRRING UPON THE 30 MINUTE SOLUBILIZATION OF NITROGENOUS CONSTITUENTS OF MUNG BEAN

Amt. Sample per 100 ml. Solvent[1]	Extraction Number	Amount of Total Nitrogen Solubilized According to Mode of Stirring	
		Mechanical	Hand
g.		%	%
5.0	1st	64.5	64.0
	2nd	10.6	8.1
	3rd	2.6	4.3
7.5	1st	61.5	61.5
	2nd	10.0	9.4
	3rd	3.0	3.7
10.0	1st	60.6	60.7
	2nd	9.2	10.5
	3rd	3.1	3.9
12.5	1st	61.0	59.0
	2nd	9.6	12.7
	3rd	3.1	4.3
15.0	1st	60.4	58.5
	2nd	9.2	12.8
	3rd	3.0	4.0
10.0[2]	1st	28.9	28.6
	2nd	20.0	19.0
	3rd	11.2	9.6

[1] The solubilizing medium consisted of 0.4 M sodium chloride.
[2] The solubilizing medium consisted of distilled water included for the purpose of comparison.

as the highest ratio yielded the least. In all instances when the extraction time was increased small increments of solubilized nitrogen were obtained. When the sample to solvent ratio was lower, the degree of solubilization was least influenced by the time of extraction. The finer ground meal gave better results.

Comparison of hand stirring with mechanical agitation (Table II) demonstrated that there was very little advantage of one over the other. Successive extractions with fresh solvent of the residue from the preceding extracts showed that there was additional solubilized nitrogen. However, it was apparent that two successive extractions were sufficient to remove practically all the dispersible nitrogen.

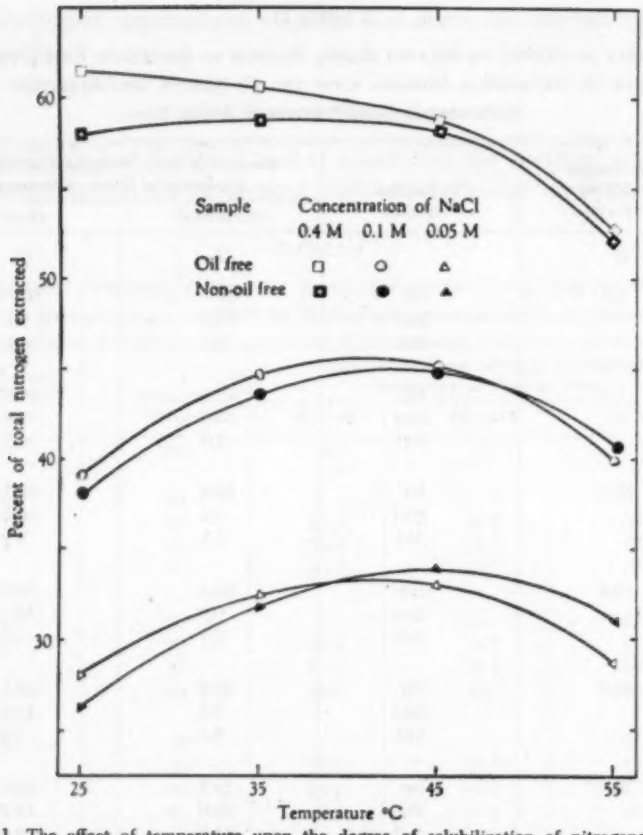

Fig. 1. The effect of temperature upon the degree of solubilization of nitrogenous compounds from oil-free and non-oil-free Mung bean meal.

In general, lipide-free samples yielded slightly higher solubilization values at the same temperature than those that were not lipide-free. However, extractions of either sample at temperatures above 45°C. produced diminished yields with lower salt concentrations. The downward slopes of all the curves, Fig. 1, beyond 45°C. indicate the possibility of heat alteration and the consequent altered solubility of thermolabile proteins. The presence of lipide seemed to contribute some protection against heat effects when using solvents of lower salt concentrations, as similarly noted by Boyer, *et al.* (1).

Increased hydrogen ion activities of the extract with pH less than 6 repressed the 0.4 *M* sodium chloride dispersibility of the nitrogenous material but not to the same extent as in the absence of the salt. From Fig. 2 it may be seen that the range of minimum solubility occurred in either case at the pH region of the isoelectric points of the proteins (4).

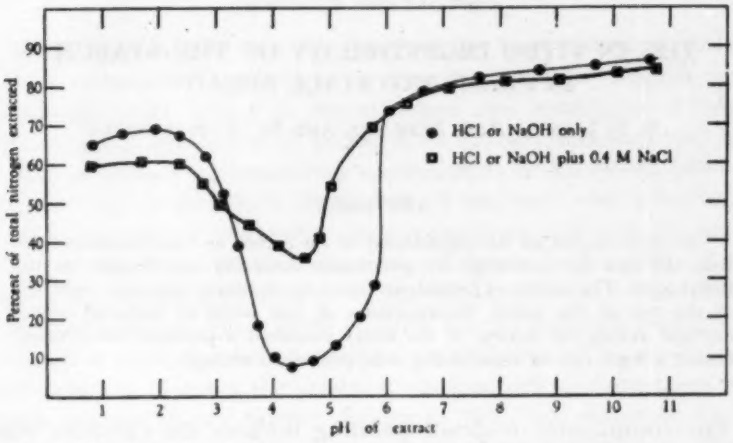

Fig. 2. The effect of hydrogen ion activity upon the salt solubilization of nitrogenous compounds of Mung bean meal.

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THE IN VITRO DIGESTIBILITY OF THE STARCH IN FRESH AND STALE BREAD[1]

S. S. JACKEL, A. S. SCHULTZ, AND W. E. SCHAEDER[2]

ABSTRACT

In vitro studies on the digestibility of the starch in bread demonstrated that the rate of hydrolysis by pancreatin decreased significantly as the bread aged. The extent of hydrolysis, however, remained constant regardless of the age of the bread. Incorporation of low levels of bacterial alpha-amylase during the baking of the bread produced a product which maintained a high rate of digestibility over prolonged storage.

The considerable evidence pointing towards the causative effect of starch retrogradation and recrystallization in the staling of bread has been reviewed recently by Geddes and Bice (5), Kerr (10), and Whistler (18). It appears well established from these data that bread staling is accompanied by changes which reverse the effect of the baking process in gelatinizing and solubilizing the starch molecules. Although *in vitro* studies by Schultz and Landis (15), Volz and Ramstad (17), and Jackel, Schultz, Schoonover, and Schaeder (9) indicate that the starch in aged bread is less susceptible to amylolytic hydrolysis than that in fresh bread, *in vivo* experiments conducted with human subjects by Habs (6) and with rats by Abelin and Biderbost (1) suggested that utilization of the carbohydrates was essentially quantitative regardless of the age of the bread.

The present study was undertaken to evaluate the effect of the age of white bread on the rate and extent of *in vitro* digestibility of the starch fraction by pancreatin. It was considered possible that staling might function to decrease the rate of digestibility without otherwise affecting the extent of digestibility. It was also the purpose of the investigation to determine how the *in vitro* digestibility of the starch in experimental breads containing bacterial alpha-amylase compared with that of conventional breads during prolonged storage. It was anticipated that differences might exist in view of previous data (16) indicating that supplementation with this enzyme retarded the development of the usual staling changes in susceptibility of the crumb starch to beta-amylase.

[1] Manuscript received December 12, 1952.
[2] The Fleischmann Laboratories, Standard Brands Inc., New York 51, New York.

Materials and Methods

The white breads[3] used in these studies were obtained unsliced from a commercial bakery unit and were stored in moisture proof cellophane at 30° and 8°C. Formulations and conditions of baking were described previously by Jackel, *et al.* (9) and Schultz, *et al.* (16).

The procedure for measuring susceptibility of the bread starch to enzymatic action described previously (9) was used with substitution of 35 or 500 mg. of pancreatin[4] for beta-amylase and with the substitution of 10 ml. of McIlvaine's citric-phosphate buffer, pH 7.5, for the pH 5.2 buffer. Briefly, 20 g. of interior bread crumb were shaken with 5 g. of bakers' yeast at 30°C. until the fermentable sugars were removed as indicated by no further gas evolution. The enzyme was then added to convert the starch to fermentable carbohydrates and the gas evolved during further shaking at 30°C. was measured by displacement in a gas burette. The amount of gas evolved was related stoichiometrically to the amount of fermentable carbohydrate produced by the enzyme action.

Results and Discussion

Effect of Age of Bread on Rate of Digestibility. The effect of age of the bread and temperature of storage on susceptibility of the starch to hydrolysis by 35 mg. of pancreatin is shown in Fig. 1. This amount of enzyme is insufficient to digest the quantity of starch present (about 8 g.) in the 2 to 5 hour hydrolysis period used. It is evident that under these conditions the rate of *in vitro* digestibility of the bread starch decreased significantly as the bread aged. Storage at 8°C. accelerated development of the increase in resistance to pancreatin action as compared to storage at 30°C. This is in agreement with previous reports (4, 9, 15) that staling is more rapid at lower temperatures. It may be concluded from these data that increased staleness of the bread, produced by aging and accelerated by storage at refrigerator temperatures, resulted in a decreased rate of *in vitro* starch digestibility. Evaluation of the significance of this finding in terms of human nutrition will not be possible until suitable *in vivo* studies are carried out. The techniques used by Rostorfer, Kochakian, and Murlin (13) in their studies on the digestion of bread in the human stomach appear applicable.

It is necessary to reconcile these results, indicating that the rate of

[3] We are indebted to Mr. F. D. Schoonover for making the breads available.
[4] U.S.P. grade, Merck and Co., Rahway, N.J.

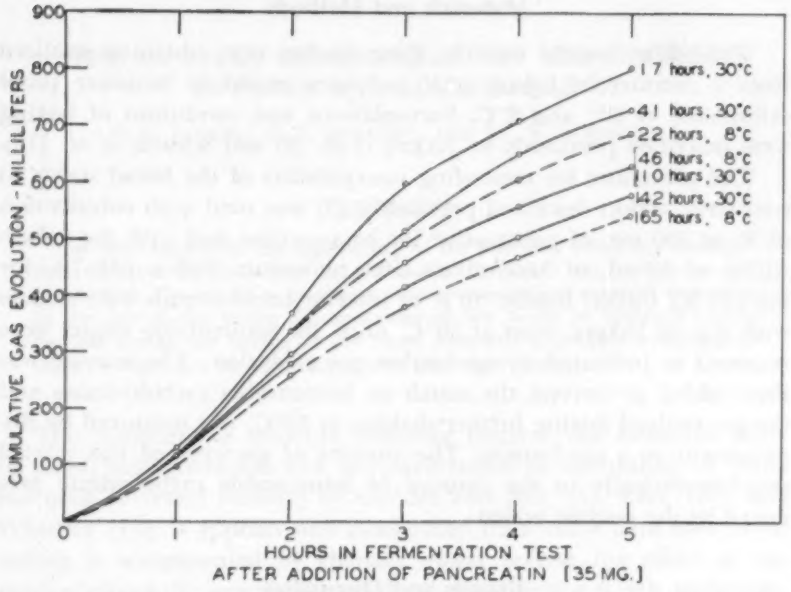

Fig. 1. Effect of time and temperature of storage of bread on rate of starch digestibility.

starch hydrolysis by pancreatin decreases as the bread ages, with other reports (2,7,8) that oven-fresh bread is attacked with difficulty by proteolytic and diastatic enzymes under *in vitro* conditions. Although the conclusions appear contrary, it is more than likely that the difference refers not to inherent susceptibility of the starch substrate to enzymic action but rather to the over-all availability of the bread sample to enzymatic attack. Oven-fresh bread does not disperse as easily as does bread only a few hours older, and there is some evidence (12) that oven-fresh bread requires very thorough mastication to ensure good digestibility. The particular problem of oven-fresh bread is a special one with only narrow implications since commercial bread is generally not less than 8 to 12 hours old when it reaches the consumer. All samples were thoroughly dispersed in our experiments by mechanical means in order to permit conclusions referring specifically to the susceptibility of the starch component to enzymatic digestion. The samples in the studies reported in the literature were not well dispersed and presumably the data obtained reflect differences in dispersibility of the sample on aging.

Effect of Bacterial Alpha-Amylase in Retarding Decrease in Rate of Digestibility on Aging. The incorporation of low levels of bacterial

alpha-amylase (3 to 6 units[5] per pound of bread) in the production of bread on a commercial scale has been reported recently by Schultz, Schoonover, Fisher, and Jackel (16). This bread is normal in all respects but it is characterized by a reduced rate of decrease in starch susceptibility to beta-amylase. The nature of the mechanism involved in the favorable action of this enzyme is not altogether clear and is being studied further. The most reasonable explanation at present is based on the known high thermostability of alpha-amylases from bacterial origin. Although cereal amylases are somewhat more stable than fungal amylases both are inactivated rapidly at temperatures equal to or slightly above the gelatinization temperature of wheat starch (63°–65°C.). It is likely that the bacterial enzyme is the principal amylase present for any appreciable length of time after the starch begins to gelatinize and is accessible to attack. Presumably the enzyme functions, via hydrolytic activity, to result in an increased proportion of low molecular weight starch degradation fragments characterized by decreased rates of retrogression. It is also probable that traces of the enzyme remain active after baking and function during storage to retard development of the associative changes which result in decreased starch susceptibility to digestive action.

That incorporation of bacterial alpha-amylase in white bread at the level of 6 SKB units per pound of bread was effective in maintaining ease of digestibility of the bread by pancreatin is evident from the data of Fig. 2. Over prolonged storage at both 30° and 8°C., gas production in the fermentation test, indicative of susceptibility of the starch to pancreatin digestion, was maintained at levels significantly higher than in normally staling control breads.

Effect of Age of Bread on Total Digestibility. It appears to be well established that the utilization of the carbohydrates in bread is essentially quantitative regardless of the age of the bread. Habs (6) concluded from studies with normal and diabetic human subjects that fresh and stale breads (wheat and rye) were equally well assimilated, and Abelin and Biderbost (1) showed in experiments with rats that the absorption of carbohydrates from white or whole wheat bread was approximately 98%. Such results would be expected from the classical studies of Langworthy and Deuel (11) indicating that man can digest and assimilate large quantities of raw starch. More recently, however, Beazell, Schmidt, and Ivy (3) have questioned this concept presenting data indicating that under *in vitro* conditions raw potato starch is entirely refractory to the action of gastro-intestinal

[5] Expressed in terms of the units of alpha-amylase activity proposed by Sandstedt, Kneen and Blish (14).

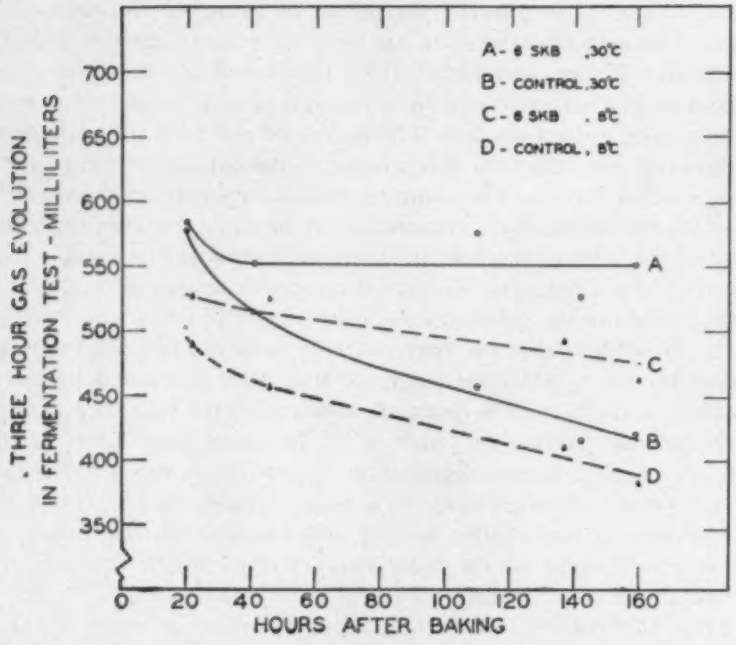

Fig. 2. Effect of supplementation with bacterial alpha-amylase (6 SKB units per pound of bread) on starch digestibility of bread stored at 8° and 30°C.

enzymes. The same authors concluded from *in vivo* experiments with human subjects that bacterial fermentation in the intestinal tract, rather than assimilation, accounted for an important part of the starch that was decomposed during passage through the alimentary tract.

In view of the known retrogradation of starch during the staling of bread, representing a polymerization and return towards the non-gelatinized state, it appeared advisable to establish whether the total amount of starch digestible *in vitro* by pancreatin decreased during the aging of bread. The same technique used to obtain the other data of this paper was followed except that the amount of pancreatin was increased to 500 mg. to provide a rapid rate of hydrolysis. From 20 g. of interior bread crumb 1220 ml. of gas ($\pm 2\%$) were obtained regardless of the age of the bread (17 to 106 hours) or the temperature of storage (8° or 30°C.). The digestion was finished in all cases in 7 hours and 90% or more of the gas evolution with each bread occurred in the first 3 hours. Additional increments of yeast and enzyme, equivalent to the initial additions, were added at the end of

the fourth hour to establish that the tapering off of the fermentation resulted from exhaustion of substrate rather than from inactivation of the yeast or the pancreatin. It may be concluded from these data that there is no difference between fresh and stale bread with respect to extent of carbohydrate digestion by pancreatin.

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AN ALKALINE WATER RETENTION CAPACITY TEST FOR THE EVALUATION OF COOKIE BAKING POTENTIALITIES OF SOFT WINTER WHEAT FLOURS[1]

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ABSTRACT

An alkaline water retention capacity test for cookie quality evaluation of soft winter wheat flours is described. Results of this test may be directly correlated with cookie diameters in contrast to those of other physicochemical methods which require adjustments for protein and/or ash content when making intervarietal comparisons. A correlation coefficient of —0.847 was obtained for alkaline water retention capacity vs. cookie diameter for 506 test samples.

In the quality characterization of soft wheat flours, results of such physicochemical tests as no-time acid viscosity and mixogram area are often used to give an indication of baking potentialities. Recently, an "Alkaline Viscosity" method was presented (3) in which the viscosity of an alkaline flour suspension was determined.

In this method a pH approximating that of chemically leavened doughs and baked products was obtained by use of a sodium bicarbonate solution. The results of this test, after adjustments are made, are highly correlated with cookie diameter. Finney and Yamazaki (2) reported on an acid water retention capacity test for hard wheat flours which correlated highly with bread loaf volume. In this test flour was hydrated in an excess of lactic acid solution and then centrifuged under specified conditions. The ability of the hydrated flour to retain water against centrifugal force was a measure of the loaf volume potentiality of a flour.

This paper describes a test that combines certain features of both the alkaline viscosity test and the acid water retention test, and thereby gives results that are correlated directly with cookie diameter, thus requiring no adjustment for protein content. In addition, alkaline water retention data are presented for flours from pure wheat varieties covering a wide range in quality characteristics and grown over a period of six crop years at several locations, and representing a wide range in protein content.

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Materials and Methods

The following procedure was used in the determination of alkaline water retention capacity: Weigh 15 g. of flour (14% moisture basis) into a weighed 100 ml. centrifuge tube with stopper. Add 50 ml. 0.1 *N* sodium bicarbonate solution, stopper, and shake vigorously to suspend flour. Remove stopper, and add 25 ml. more of the same solution. Stopper, mix solution, and start timer. Thereafter, shake tube vigorously and allow to stand for 20 minutes, shaking every 5 minutes. Remove stopper, centrifuge for 15 minutes at 1800 r.p.m. (International Centrifuge No. 2), decant off supernatant liquid, and drain tube for 10 minutes at an angle of 10 to 15°. Restopper and weigh tube with contents. The gain in weight is expressed in percent.

The method was developed using composites of eight pure soft wheat varieties and further tested in duplicate on a total of 506 samples of 11 named varieties covering the range of wheat quality from hard winter to soft white grown from 1944 to 1949, inclusive, and representing a wide range in protein content (5.7 to 14.0%). The following locations were represented in the samples used, either as individuals or as parts of composites: Urbana, Ill.; Lafayette, Ind.; Thayer, Kans.; Lexington, Ky.; Beltsville, Md.; East Lansing, Mich.; Columbia, Mo.; Ithaca, N.Y.; Waynesville, N.C.; Columbus, O.; Germantown, O.; Holgate, O.; Wooster, O.; Stillwater, Okla.; Knoxville, Tenn.; Morgantown, W. Va.; and Madison, Wis.

Experimentally milled flours were obtained by means of the Buhler mill, using procedures that are standard at the Soft Wheat Quality Laboratory. Cookies were baked from these samples following the method described by Finney, Morris, and Yamazaki (1).

Results

Preliminary experiments in which conditions of the test were varied indicated the following: (a) Water-flour ratios of less than five gave ranges which were considered to be too narrow, whereas greater ratios necessitated a reduction in the weight of flour used, again resulting in narrow ranges. (b) Longer centrifuge times appeared to distort results since samples had different packing or compression rates, and shorter times resulted in incomplete sedimentation in some cases. (c) The concentration of sodium bicarbonate solution used could be varied somewhat without affecting the outcome greatly, and the period of contact of alkaline solution and flour was not of importance as long as the flour was completely wetted. (d) Addition of the reagent in two steps was necessitated by the fact that the volume of the flour prior

to wetting was so great that uniform suspension of flour was impeded when all of the solution was added at one time.

The quantity of nitrogenous materials extracted by the alkaline solvent (pH of supernatant liquid = 8.0 to 8.1) under the conditions of the test varied from about 17 to 29% of the total nitrogen depending on flour protein content and variety. Although there were definite indications that the amount of soluble nitrogen was related to variety within a given flour protein range, the relationship appeared to have no bearing on cookie baking characteristics.

Averaged alkaline retention results obtained on the 506 samples (standard error of replication (s) = 0.50% for n = 2) were correlated with cookie diameter (sum of diameters of two cookies) in order to determine the extent of association and degree of predictability. Table I presents statistical data relative to the comparison.

TABLE I

STATISTICAL DATA FOR THE RELATIONSHIP BETWEEN ALKALINE WATER RETENTION CAPACITY (X) AND COOKIE DIAMETER (Y)

Year	n	r =	Regression Equation $\hat{Y}$ =	$S_{y.x}$
				cm. x 2
1944	56	−0.778[1]	−0.112X + 22.91	
1945	88	−0.863	−0.112X + 22.77	
1946	98	−0.892	−0.121X + 23.26	
1947	66	−0.881	−0.130X + 24.41	
1948	99	−0.855	−0.122X + 23.72	
1949	99	−0.936	−0.118X + 23.63	
1944-1949	506	−0.847	−0.127X + 23.86	0.426

[1] All correlation coefficients are significant beyond the 0.1% point.

The very highly significant correlation coefficients, both for any single crop year and for all seasons combined, indicate that alkaline water retention capacity is an index of the cookie baking potentialities of flours regardless of seasonal or individual sample variations.

In order to determine the extent to which variety accounts for differences in response to this test, 6-year varietal means were plotted against mean cookie diameters (Fig. 1). The correlation coefficient of r = −0.95 was obtained for alkaline water retention vs. cookie diameter. This high degree of association together with a low standard error of estimate ($S_{y.x}$ = 0.17 cm.) indicate that the retention test is reflecting faithfully the varietal response to the cookie test. Three varieties,

Clarkan, Fairfield, and Wabash, digress somewhat from the regression line, but the diversions do not appear to affect seriously the over-all picture (Fig. 1).

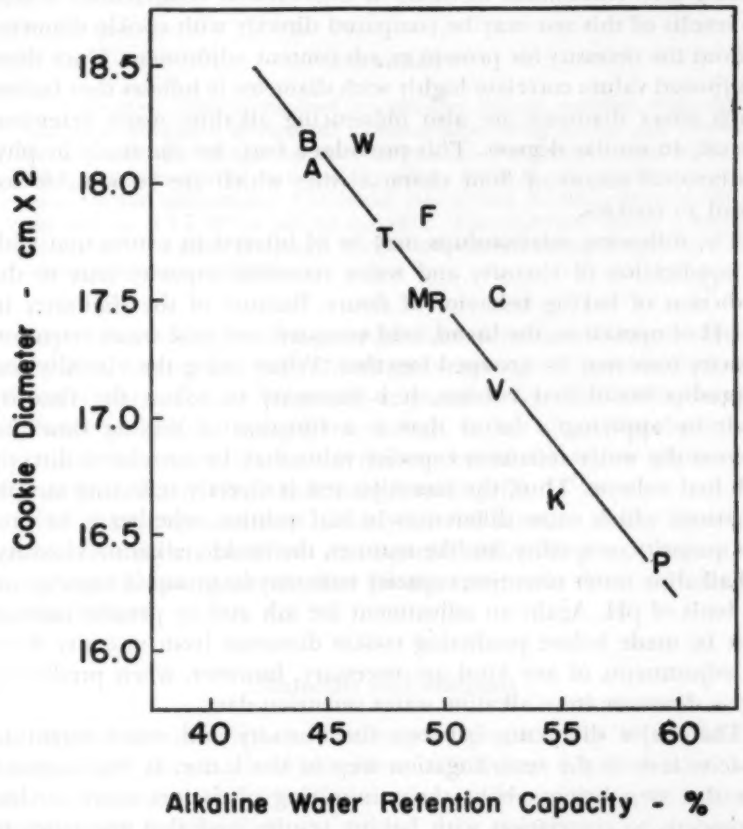

Fig. 1. Relation between alkaline water retention capacity and cookie diameter using varietal means. P = Purkof, K = Kharkof, V = Kawvale, C = Clarkan, R = Trumbull, M = Minturki, F = Fairfield, T = Thorne, W = Wabash, A = American Banner, and B = Blackhawk.

Discussion

It has been shown by several writers, especially Sharp and Gortner (4), that when wheat flour is suspended in aqueous media of varying pH, hydration of gluten takes place in acid solutions while in moderately alkaline media swelling does not take place to any great extent. Most flours which have superior imbibitional properties in acid solution also behave similarly at other pH levels. There are some, however, to which this general rule does not apply. Thus, the use of a

physicochemical test in acid solution to predict baking behavior in an alkaline medium is open to question.

An advantage which the alkaline water retention test possesses over existing physicochemical methods in intervarietal comparisons is that the results of this test may be compared directly with cookie diameter without the necessity for protein or ash content adjustment. Since these unadjusted values correlate highly with diameter it follows that factors which affect diameter are also influencing alkaline water retention capacity to similar degrees. This provides a basis for the study by physicochemical means of flour characteristics which are responsible for spread in cookies.

The following relationships may be of interest in connection with the application of viscosity and water retention capacity tests to the prediction of baking behavior of flours. Because of the similarity in the pH of operation, the bread, acid viscosity, and acid water retention capacity tests may be grouped together. When using the viscosity test to predict bread loaf volume, it is necessary to adjust the viscosity result by applying a factor that is a function of mixing time (2); whereas the water retention capacity value may be correlated directly with loaf volume. Thus, the retention test is directly reflecting sample variations which cause differences in loaf volume, whether it be protein quantity or quality. In like manner, the cookie, alkaline viscosity, and alkaline water retention capacity tests may be grouped together on the basis of pH. Again an adjustment for ash and/or protein content must be made before predicting cookie diameter from viscosity data. No adjustments of any kind are necessary, however, when predicting cookie diameter from alkaline water retention data.

The major difference between the viscosity and water retention capacity tests is the centrifugation step in the latter. It thus appears that this step brings about the minimizing of factors more or less extraneous to correlation with baking results, and that resistance to removal of solvent, or lack thereof, is more closely associated with wheat quality than free hydration or sorption *per se*.

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KINETIC STUDIES OF THE LIPOXIDASE SYSTEM OF WHEAT[1]

G. N. IRVINE AND J. A. ANDERSON

ABSTRACT

Lipoxidase extracts obtained by grinding durum semolina with sand and water have been investigated manometrically using linoleic acid suspensions as substrate. Oxygen uptake follows a two phase reaction and rates of both phases have been measured at various levels of pH, oxygen, enzyme, substrate and temperature. The initial rate follows Michaelis-Menton kinetics and K_m is 5×10^{-6} at pH 6.5 and 30°C. The activation energy between 10 and 30°C. is 6,500 calories. A routine manometric assay for lipoxidase in wheat products is outlined.

Lipoxidase catalyzes the peroxidation of certain unsaturated fatty acids. The enzyme has been shown to be present in various flour fractions (3) where it may be partly responsible for the development of oxidative rancidity during flour storage. It has also been shown that lipoxidase is responsible for the destruction of the yellow pigments of durum semolina during macaroni processing (2).

The work reported here deals with the kinetics of the lipoxidase system as it occurs in wheat endosperm, and with an attempt to develop a simple manometric assay for the enzyme in flour, semolina, or ground wheat.

Materials and Methods

Preparation of Enzyme. The enzyme preparations were obtained from various durum wheat semolinas milled from pure varieties. Most of the work was done with two varieties which are rich in this enzyme, Pelissier and Golden Ball.

Active enzyme solutions were prepared by grinding 10 g. of semolina with 5 g. of sand in 20 ml. of distilled water in a mechanical mortar grinder for 3 minutes. The mass was transferred to a 50 ml. round bottom centrifuge tube and spun for 10 minutes at top speed in a clinical centrifuge. The supernatant constituted the enzyme extract. Processed in this way, extractions made from the same sample of material are highly reproducible from day to day. Prior extraction

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of lipids did not significantly increase or alter the activity of the extracts.

Preparation of Substrate. The substrate employed was linoleic acid which was available in two grades, one a 60% linoleic acid, the other a pure linoleic acid obtained from the Hormel Foundation, Austin, Minnesota. All the experiments reported here were made with substrate suspensions of the 60% acid. But the observations were compared from time to time with similar experiments using suspensions of the pure acid, to ensure that none of the phenomena observed were due to the impurity of the 60% substrate.

Since the pH optimum for wheat lipoxidase is about 6.5, an emulsified preparation of linoleic acid must be used. An extremely stable emulsion has been prepared using Triton X-100[2], a surface active agent which has no effect on the enzyme system. Two millilitres of Triton X-100 were added to 10 ml. of the 60% linoleic acid and water was added slowly with stirring until the emulsion inverted; sufficient water was then added to make up to 250 ml. The resulting preparation was then mixed for 3 minutes at high speed in a Waring Blendor. This emulsion showed no tendency to separate out if kept in a refrigerator for as long as a month, and there was no significant autoxidation during this time.

Emulsions prepared from the 60% acid showed no significant induction period during oxygen uptake measurements, the pure acid so prepared did show a short induction period; accordingly the pure acid was used only as a check on the results obtained with the 60% acid.

Method. The method used to follow the kinetics was the direct manometric method of Warburg, which measures the uptake of oxygen by mixtures of enzyme and substrate in buffered solutions.

Oxygen uptake measurements were made in conventional Warburg flasks of approximately 15 ml. capacity. For experiments where greater uptakes were desired than could be attained with these flasks, 50 ml. flasks were used. Unless otherwise noted, all experiments were run with an atmosphere of oxygen in the flasks, obtained by venting with oxygen for 10 minutes before mixing the reactants. Shaking speed was 130 oscillations per minute at an amplitude of 4 cm. The flasks were allowed to equilibrate for a few minutes after the oxygen had been run in, the stopcocks were then closed and the contents of the sidearm tipped into the main compartment; time was reckoned from the moment of tipping. Except where indicated, the water bath was maintained at 30.0°C.

[2] A nonionic surface active agent obtainable from Rohm and Haas Co., Philadelphia.

Results

Typical oxygen uptake curves are shown in Fig. 1. Curve A represents a condition of high enzyme concentration and very low substrate concentration; it is roughly first order and flattens at an uptake corresponding to one mole of oxygen per mole of acid. Curve B represents all other combinations of enzyme and substrate levels; this is the type of curve which is almost invariably obtained. It is a two stage reaction curve with a rapid initial uptake whose velocity falls off in approximately a first order manner and then reaches a steady-state or zero order phase where the rate is constant. This zero order stage has been followed beyond an uptake of two moles of oxygen per mole of acid with only slight diminution of the rate.

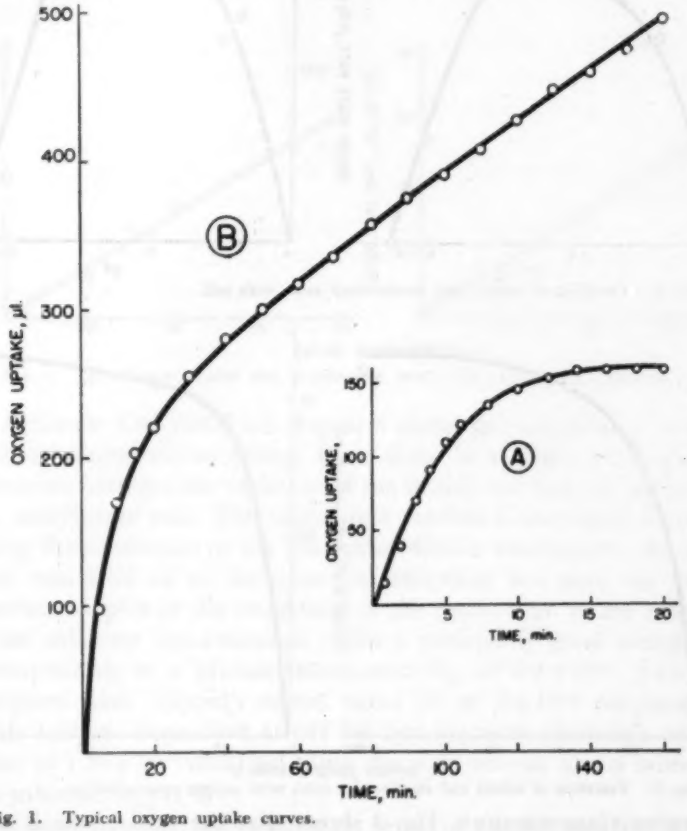

Fig. 1. Typical oxygen uptake curves.

Because of the two distinct stages of the reaction, measurements have been made of both the initial rate and the steady-state rate.

Hydrogen-ion Concentration. Variations of the initial and steady-state rates with pH are shown in Fig. 2. The optima for both rates appear to be about pH 6.5, and this was chosen as the pH for all other experiments. Süllmann, working with soybean lipoxidase (4) reported a similar pH optimum. But Holman, using the crystalline soybean enzyme with homogeneous substrate solutions (1), gives the optimum at pH 9; he suggests, however, that this pH may not be the physiological optimum.

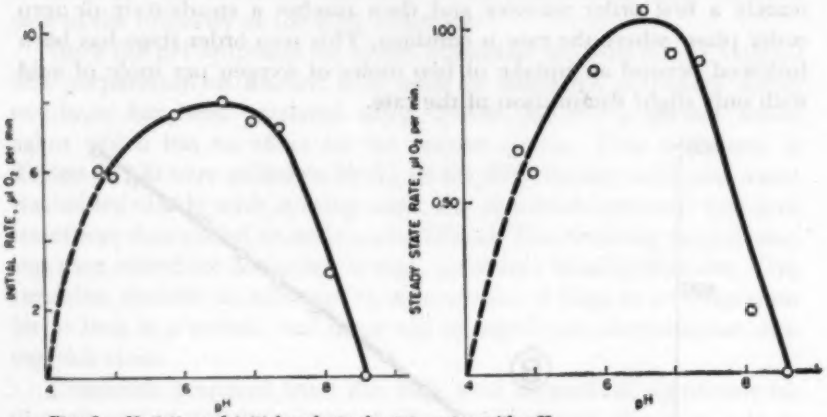

Fig. 2. Variation of initial and steady-state rates with pH.

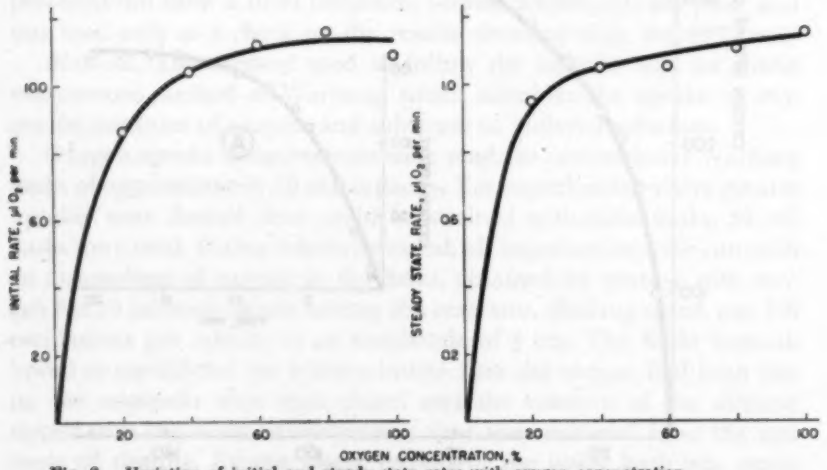

Fig. 3. Variation of initial and steady-state rates with oxygen concentration.

Oxygen Concentration. Fig. 3 shows that the initial rate is somewhat more sensitive to oxygen concentration than is the steady-state

rate. The latter has reached a maximum at the level of air (20%), while the initial rate reaches a maximum beyond 40%. Accordingly, for concentrations above about 40% oxygen, both rates are virtually independent of the oxygen concentration.

Enzyme Concentration. The effect of enzyme concentration on the rates at a reasonably high substrate level is shown in Fig. 4. At this substrate level, the rates of both the initial and steady-state reactions are linear with respect to enzyme concentration and are also proportional to one another. Thus the steady state rate is a simple linear function of the initial rate for a given preparation. A plot of the log of initial rate against log of enzyme concentration yields a straight line with a slope of unity; this indicates that the reaction is first order with respect to enzyme concentration.

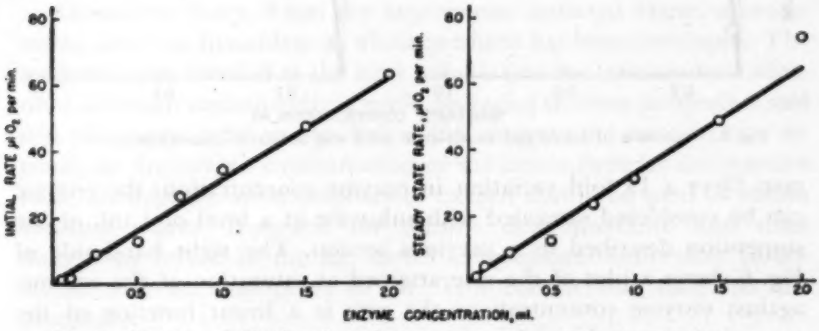

Fig. 4. Variation of initial and steady-state rates with enzyme concentration.

Substrate Concentration. Figure 5 shows the variation of the rates with substrate concentration. Here there is a slight but significant difference between the variation of the initial rate and the variation of the steady-state rate. The *initial rate* reaches a saturation value, fulfilling the conditions of the Michaelis-Menton mechanism; the steady-state rate falls off at the same concentration but does not become constant. A plot of the reciprocal of the *initial rate* vs the reciprocal of the substrate concentration yields a reasonably good straight line corresponding to a Michaelis constant, K_m, of 5.0×10^{-6}. This value compares with Tappel's recent value (5) of 2×10^{-5} obtained with crude soybean lipoxidase at pH 9.0 but not with Holman's previous value of 1.35×10^{-3} obtained using the pure enzyme and a homogeneous substrate at pH 9.

A factorial experiment using six levels of enzyme and five levels of substrate yielded the data shown on the left in Fig. 6, for the initial

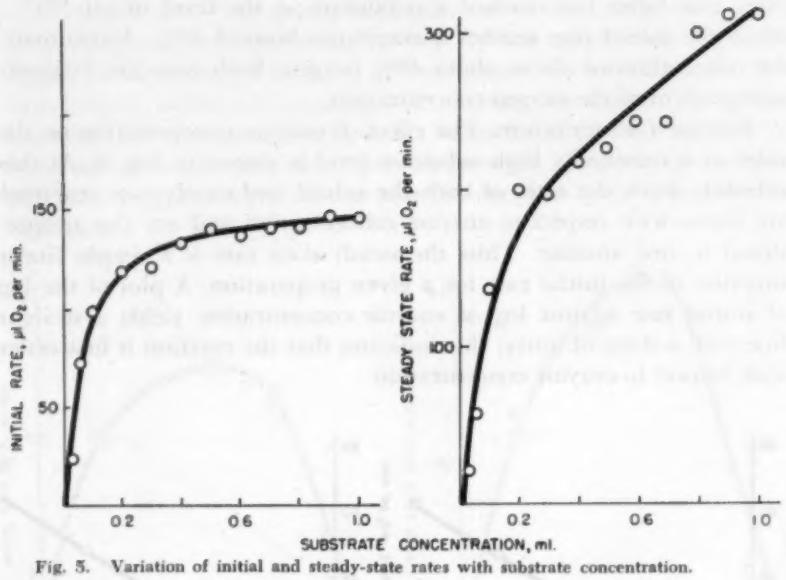

Fig. 5. Variation of initial and steady-state rates with substrate concentration.

rate. Over a 15 fold variation in enzyme concentration, the enzyme can be considered saturated with substrate at a level of 1 ml. of the suspension described in a previous section. The right hand side of Fig. 6 shows a plot of the rate attained at saturation of the enzyme, against enzyme concentration; the rate is a linear function of the enzyme concentration.

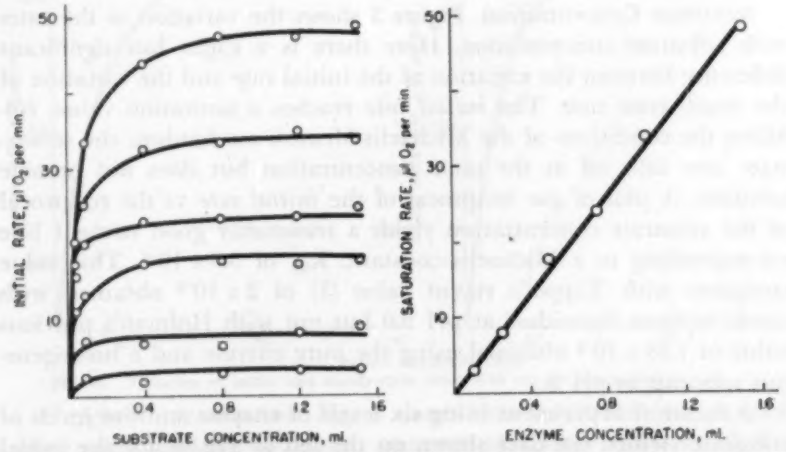

Fig. 6. Variation of initial rate with substrate concentration at various enzyme levels (left). Variation of initial rate at saturation with enzyme concentration (right).

Temperature. The effect of temperature on the enzyme system is complex. Direct temperature inactivation of the enzyme is not apparent below about 60°C.; incubation of the enzyme extract for 2½ hours at 60°C. resulted in 42% inactivation of the enzyme. In the range between 10 and 50°C. there is a very marked effect of temperature on the relative rates of the two stages of the reaction. A full discussion of this phenomenon will be published in a subsequent paper. The activation energy for the initial rate was determined in the range 10-30°C. and yielded a value of 6,500 calories. This is somewhat higher, but of a similar order to Tappel's value of 4,200 calories (5). Since the initial rate at 30°C. fell on the Arrhenius line for the range 10-30°, and since this is about the temperature used in both bread dough and macaroni dough processing, 30°C. was chosen as the standard for the work reported in this paper.

Lipoxidase Assay. From the experiments outlined above, a manometric assay for lipoxidase in wheat products has been developed. The method is put forward as the most reliable one for routine determinations although undoubtedly it could be varied in some particulars and still yield very useful results. Below 40% oxygen, the reaction rate depends on the oxygen concentration of the atmosphere in the reaction flask; accordingly, an atmosphere of oxygen should be used to obtain absolute results. However, for routine determinations, and since doughs are mixed in the air, useful comparative results may be obtained with an atmosphere of air. This considerably simplifies the procedure.

The optimum pH is about 6.5 and, since this is similar to the pH of wheat doughs, a phosphate buffer 0.067 molar of pH 6.5 is used.

The enzyme and substrate preparations are made up as described in the earlier section on methods; 1.0 ml. of substrate is used in the body of the reaction vessel, and 0.8 ml. of the enzyme preparation is used in the sidearm of the flask. With 15 ml. flasks a convenient total volume is 3.8 ml., and 2.0 ml. of buffer are therefore pipetted into the main compartment along with the substrate. At this substrate level, the enzyme in 0.8 ml. of extract will undoubtedly be working at the saturation level; this is apparent from Fig. 6. Either the initial rate or the steady-state rate may be used as the measure of enzymic activity; the choice is arbitrary. A reading of the uptake at 5 minutes reaction time is a convenient measure of the initial rate but this result will depend upon a single reading. The steady-state rate should give a somewhat more reliable reading as it is based on a number of observations at various time intervals, but its measurement is a much more

time-consuming process, and appreciable errors may arise in determining the slope of the line. From a theoretical standpoint, the measure of the initial rate is preferable; accordingly the measurement of the oxygen uptake at 5 minutes has been adopted as the measure of lipoxidase activity for ground wheat, flour, or semolina.

Discussion

The results obtained in this study are generally consistent with those obtained by other investigators working with a similar method on lipoxidase preparations from other sources. The lipoxidase system, at least as it occurs in nature, appears to be a complex one. Preparations of the enzyme from different sources will vary widely in the level of lipoxidase activity and also in the additional components of the system which may be present. Accordingly, different investigators will be working with quite different levels of enzyme and substrate.

From the point of view of a mechanism for the lipoxidase oxidation, the principal feature observed in this work has been the two distinct phases of the reaction. This feature has not been emphasized by previous workers and in some cases was not observed owing to the methods used. Süllmann's work (4) on oxygen uptake of soybean lipoxidase extracts clearly shows this two-phase characteristic. But, owing to the high activity of his extracts, the initial phase appears on a somewhat smaller scale than in the present investigation, and his reaction curves indicate principally the second phase. He does not discuss the two distinct phases.

It has generally been noted that the activity of the extracts falls off with time, and the implication has been that the enzyme is inactivated in some way. This might be true if the activity fell off continuously after the initial phase. However the development of a steady-state rate following the initial rapid phase would seem to preclude this explanation. It appears possible that the variation of the rates of the two phases of the reaction with temperature may provide a key to the mechanism, and work is going ahead on this facet of the investigation.

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NOTE ON THE LIPOXIDASE ACTIVITY OF VARIOUS NORTH AMERICAN WHEATS[1]

G. N. IRVINE AND J. A. ANDERSON

ABSTRACT

Relative lipoxidase activities have been determined for 16 wheat varieties. There is some indication that the red wheats (hard spring, hard winter, and soft winter), as a class, have higher activities than the white wheats and the newer varieties of amber durum wheat. Results ranged from 40μl of 0_2/min./g. for Marquis and Golden Ball to 14 for Mindum and 12 for Nugget.

The presence of lipoxidase in wheat endosperm was first reported by Miller and Kummerow (3). Using decolorization of carotene solutions as a measure of the lipoxidase activity, they found that the lipoxidase activity of wheat was greatest in the germ fraction and least in the patent flour fraction.

Lipoxidase, in the form of soybean meal has long been used in the baking industry for flour bleaching, and a patent covering the preparation of the additive and its use in bread doughs was issued to Haas and Bohn in 1934 (1). The natural bleaching of the carotenoid pigments in flour during the preparation of bread doughs, especially during mixing, is undoubtedly due to the presence of lipoxidase in the flour. Yet the work of Miller and Kummerow suggested that flour contained comparatively little lipoxidase; this probably results from their comparing the activity with soybean meal which is the richest source known.

Studies in this laboratory (2), concerned mainly with durum semolina, served to develop modified methods of extracting the lipoxidase and of determining its activity by a direct manometric measurement

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of the oxygen consumed during the oxidation of a linoleic acid substrate. Casual tests of bread flours by these methods showed greater comparative activity than had previously been suspected. An opportunity was therefore made to compare the lipoxidase activities of typical varieties representing several classes of wheat. This preliminary exploration of the range of lipoxidase activity in wheat varieties is reported in this paper.

Materials and Methods

The wheats used for this study were obtained from various agricultural stations and represented pure varieties. Four varieties of hard red spring, four amber durum, two soft red winter, two hard red winter, and four white wheat varieties were included. The common wheats were milled in the laboratory to yield a straight flour of 70% extraction; the durums yielded a semolina of 50% extraction. Enzyme extracts were prepared from these materials (2).

The lipoxidase activity was measured manometrically using a procedure previously described (2). The activity figure given in Table I represents the oxygen uptake in microliters per minute, during the first five minutes of reaction, per gram of flour or semolina.

Results

The lipoxidase activities of the various flours or semolinas are given in Table I. As with most other properties of wheat, lipoxidase activity varies both with variety and with environment. Accordingly the values given in this Table will permit only general deductions. As a class, the red wheats appear to show a considerably higher lipoxidase activity than the white wheats or perhaps the durums. Golden Ball, a very poor durum variety for macaroni-making purposes, ranks with the red wheats; whereas Mindum, and the two newer varieties of durum wheat, Carleton and Nugget, rank with the white wheats. These last three durum varieties, all of which have excellent macaroni-making quality, may indicate how a breeding program, aimed almost exclusively at producing varieties which retain their carotenoid pigments during macaroni processing, can radically alter the characteristics of new members of a class of wheat. Two of the white wheats, both white springs, compare with the best durums for low lipoxidase activity; accordingly these varieties might prove useful when "filler wheats" must be used in blends for macaroni-making purposes because of shortages of durum wheat. The results also show

TABLE I

LIPOXIDASE ACTIVITY OF FLOUR OR SEMOLINA MILLED FROM VARIOUS NORTH AMERICAN WHEAT VARIETIES

Class of Wheat	Variety	Lipoxidase Activity
		$\mu l\ O_2/min./g.$
Hard red spring	Marquis	40
	Garnet	37
	Thatcher	36
	Redman	32
Hard red winter	Pawnee	38
	Commanche	22
Soft red winter	Vigo	38
	Thorne	38
White wheat	Golden	26
	Elmar	22
	Baart	16
	Idaed	14
Amber durum	Golden Ball	40
	Carleton	14
	Mindum	14
	Nugget	12

that admixtures of quite small amounts of the red wheats with high quality durum wheats are likely to have a highly injurious effect on the macaroni-making quality. The need for careful selection of varieties for macaroni-making is thus emphasized.

Acknowledgment

The authors are grateful to Mr. C. C. Fifield of the U.S.D.A. who arranged to supply them the soft red winter, hard red winter and white wheat samples.

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EFFECTS OF DRIED WHEY AND VARIOUS SUGARS ON THE QUALITY OF YELLOW CAKES CONTAINING 100% SUCROSE[1,2]

JACQUELINE DE GOUMOIS AND FLORA HANNING

ABSTRACT

The addition of 30% sucrose, glucose, alpha lactose, or beta lactose to a basic cake formula containing 100% sucrose resulted in increases in volume, compressibility, and judges' scores for texture and tenderness. The scores for grain, however, were not improved by the addition of any of the sugars. The cakes which had additions of sucrose or beta lactose had larger volumes, and those which contained beta lactose were the most compressible throughout a storage period of 5 days. Crust color varied greatly, the darkest color being obtained when glucose was included in the formula, and the lightest color when sucrose was the only sugar used.

When 15% of these same sugars was added to the basic formula and batter viscosity adjusted, the effects were, in general, the same, but the changes were not as pronounced as when the higher amounts of sugar were used. The cakes containing 20% dried whey had high compressibility ratios but showed no increase in volume as did the cakes having increased sugars. The improvement of the texture and tenderness of "whey" cakes as indicated by judges' scores was statistically significant; however, the addition of the various sugars did not show as great an improvement. Thus, there is an enhancement of softness, moistness and tenderness of cakes containing whey greater than can be accounted for by a comparable increase in lactose, glucose, or sucrose.

The addition of 10 to 15% dried whey (based on weight of flour) has been shown to improve the tenderness, texture, and keeping quality of cakes of relatively low sugar content (100%) (3). Since the lactose content of whey is high (about 73% calculated as monohydrate), part of these effects on cakes may have been due to the addition of this sugar. Therefore, the present study was undertaken to compare the effect of additions of dried whey or of various sugars to a basic cake formula containing 100% sucrose. The sugars included in the study were: sucrose, glucose, and the alpha and beta forms of lactose.

Although there have been no reports in the literature dealing with the effect of either form of lactose on cake quality, several studies have shown that sucrose included in the formula influences the volume, texture, and tenderness of cakes. Bailey and LeClerc (1) observed that

[1] Manuscript received November 21, 1952. Contribution from the School of Home Economics, University of Wisconsin, Madison, Wisconsin.

[2] Financed in part by an Industrial Research Grant from Western Condensing Company, Appleton, Wisconsin. Published with the approval of the Associate Director of the Wisconsin Agricultural Experiment Station.

cakes low in sugar tend to have large holes and a bready texture, and recommended that the level of sugar be 35% greater than that of the flour. Davis (2) reported that a 25% increase in sucrose resulted in cakes that had coarser grain, larger volumes, and better textures than control cakes which contained equal amounts of flour and sugar. Pyke and Johnson (4) reported that high-sugar-ratio cakes, containing relatively high amounts of sucrose as well as increases in shortening, eggs, and liquid, had large volumes and excellent keeping qualities.

Aside from sucrose, the only other sugar that is used to any extent in bakery products is glucose which is frequently added to bread and cakes. Bailey and LeClerc (1) observed that a 3 to 10% replacement of sucrose with invert sugar, resulted in cakes which had increased moisture retention and improved keeping qualities. This observation has been frequently repeated in the literature in connection with the use of honey, which contains approximately 75% invert sugar. However, no reports have been found of the effects of glucose itself in cakes.

The present investigation was divided into two sections. First, in order to examine the characteristic effects of substantial additions of the various sugars, cakes of a basic formula containing 100% sucrose were compared with cakes that had 30% sucrose, glucose, alpha lactose or beta lactose added to the basic formula. In the second section, cakes of the basic formula were compared with cakes that had additions of 15% of these same sugars and cakes containing 20% dried whey. This level of whey was used in order to have approximately the same total sugar content in all the cakes.

Procedure

Preparation of Cakes. The basic formula and the variations are indicated below. Cake flour, a household type of vegetable shortening, and fresh eggs were used throughout. The spray-dried whey, obtained from the Western Condensing Company, Appleton, Wisconsin, was an amorphous form and contained both alpha and beta lactose approximately in equilibrium ratio. The alpha lactose was a chemical reagent listed as 99.8% pure; and the beta lactose was made by the National Milk Sugar Company of the Borden Company and presumably contained some alpha lactose. The glucose was "Cerelose." The methods were essentially those described for all except Series 1 cakes reported in a previous paper (3). Six replications were made throughout.

Basic Formula	g.
Flour	100
Sucrose	100
Shortening	30
Eggs	40
Baking Powder	4
Salt	2
Water	85

Variations from Basic Formula

Section I

1. None
2. Sucrose—30% added
3. Glucose—30% added
4. Alpha lactose—30% added
5. Beta lactose—30% added

Section II

1. None; water reduced to 82 g.
2. Sucrose—15% added
3. Glucose—15% added
4. Alpha lactose—15% added; water increased to 86 g.
5. Beta lactose—15% added
6. Dried whey—20% added; water increased to 90 g.

Objective Tests. Batter viscosity, weight, volume, and compressibility were determined for all cakes in the manner described in the previous paper on the influence of dried whey on cake quality (3). Batter viscosity was determined with a Brabender Viscometer, volume by the seed displacement method, and compressibility was measured with a Baker Compressimeter at 3, 24, 72, and 120 hours after baking.

In addition to these tests, the differences in crust color for cakes of four replicates of Section II were measured with a Hunter Multipurpose Reflectometer. For each test, the tops of four of the cakes representing each formula were removed and cut to fit into petri dishes. These were then placed in cardboard holders which exposed only the browned surface of the sample that was the same size and shape as the window of the reflectometer. The samples were compared with a white standard of magnesium oxide taken as 100%. Measurement was made at an angle of 45° direct reflectance using a luminous green filter.

Scoring Panel. A panel of five experienced judges scored the cakes of each replicate at 24 and 72 hours after baking. For each test, one cake of each formula was cut into ½ in. slices which were 3 in. long and 2 in. wide. The judges received "blind" samples which had been wrapped in waxed paper and coded. The crusts were removed to prevent the judges from recognizing the samples through the marked differences in crust color. Therefore, only the internal crumb characteristics were judged using the following score card:

Grain	20
Texture: softness, silkiness, moistness	20
Tenderness	15
Flavor	25
Sum of Scores	80

In Section I, grain was not scored at 72 hours after baking.

Results and Discussion

SECTION I. Comparison of the effects of the addition of 30% sucrose, glucose, alpha lactose or beta lactose to the basic formula.

Batter Viscosity. As shown in Table I, the addition of 30% of the various sugars to the basic formula increased the batter viscosity. Alpha lactose resulted in the greatest increase.

Weight, Volume and Specific Gravity. There was not much variation in the weight of the cakes after baking; all lost about 16% in weight. However, as shown in Table I, the addition of any of these sugars to the basic formula increased the volume of the cakes. The addition of sucrose or beta lactose resulted in cakes of the greatest volume. The cakes containing these two sugars were light and porous and tended to be coarse in grain. Those containing alpha lactose or glucose had somewhat smaller volumes and were less porous. The cakes of the basic formula were the smallest in volume and most compact. Specific gravity varied inversely with volume, the cakes from the basic formula having the highest average specific gravity.

Compressibility. As shown in Table I, the addition of 30% of any of these sugars resulted in cakes of much greater compressibility, and these cakes retained their higher degree of compressibility throughout the storage period of five days. The addition of beta lactose resulted in the greatest increase in compressibility.

Scoring Panel. At 24 hours after baking, the judges' scores, presented in Table I, indicate that the addition of 30% of any of the sugars resulted in an improvement in texture and tenderness. The cakes which contained glucose or beta lactose received the highest scores for these characteristics. The scores for grain, however, were not improved by the addition of any of the sugars; on the contrary, the cakes which contained the lower amount of sugar received the highest scores for grain. These results are not surprising since the cakes of the basic formula were more compact, less tender, but the grain was proportionately closer and more uniform than in the cakes made with additional sugar. The flavor scores for all the cakes were quite similar.

At 72 hours after baking, the variation among samples was much less pronounced. The scores for the cakes of the basic formula showed little or no decline in texture and tenderness, but declined more in flavor than the other samples. The cakes which had sugar additions were all much alike at 72 hours after baking, and changed more in texture and tenderness than in any other characteristic. In addition, the cakes which contained beta lactose were rated higher in texture at

TABLE I

QUALITY CHARACTERISTICS OF CAKES OF SECTION I

	Basic Formula	Addition of 30% Various Sugars to the Basic Formula			
		Sucrose	Glucose	Alpha Lactose	Beta Lactose
Batter viscosity Average	567	621	648	703	636
Volume (ml.) Average	299	340	334	329	343
Specific gravity Average	0.34	0.29	0.30	0.31	0.30
Compressibility Ratios					
3 hours	0.34	0.60	0.58	0.58	0.68
24 hours	0.27	0.46	0.44	0.46	0.53
72 hours	0.20	0.37	0.35	0.33	0.39
120 hours	0.17	0.29	0.28	0.30	0.30
Average Scores 24 Hours after Baking					
Grain	17.3	15.2	16.3	15.7	15.8
Texture	15.7	16.0	16.9	16.5	16.6
Tenderness	11.8	13.4	14.0	13.2	13.9
Flavor	19.8	20.5	20.5	19.2	19.6
Sum of scores[1]	64.6	65.1	67.7	64.6	65.9
Average Scores 72 Hours after Baking					
Texture	15.4	15.1	15.7	15.1	15.7
Tenderness	12.3	13.2	13.3	12.6	13.2
Flavor	18.4	20.3	19 7	19.6	19.8
Sum of scores[1]	46.1	48.6	48.7	47.3	48.7

[1] Maximum scores in all characteristics would give a total of 80 at 24 hours and 60 at 72 hours storage period.

72 hours and in tenderness at both 24 and 72 hours after baking than those which contained alpha lactose. The cakes containing beta lactose were also larger in volume, and more compressible, thus, it would seem possible that under these conditions the two forms of lactose functioned differently in a cake batter.

In addition to the numerical scores, the judges made frequent comments about the different samples on the score sheets. The cakes of the basic formula received the greatest number of unfavorable comments, a total of 15, and were considered by many of the judges

to be dry and, although fine in grain, too compact in structure. The cakes containing glucose, alpha lactose or beta lactose received only a few comments, which were for the most part favorable. Those which contained additional sucrose, however, received more unfavorable comments, eight in all, and the most frequent remarks were concerned with the comparative dryness and coarse texture of these high sucrose cakes.

SECTION II. Comparison of the effects of the addition of 15% sucrose, glucose, alpha lactose, beta lactose or 20% dried whey to the basic formula.

Batter Viscosity. Since the addition of the various sugars or whey to the basic formula increased batter viscosity, and this factor might influence other characteristics of the cakes, the water content was varied as indicated in the procedure, in an attempt to obtain uniform batter viscosities for all samples. The resulting viscosities are presented in Table II.

Weight, Volume, and Specific Gravity. As shown in Table II, the cakes having the addition of 15% of any of the sugars, and adjustments in water content, showed the same trend on volume and specific gravity as when 30% of these same sugars were added to the basic formula. All cakes lost about 16% in weight during baking. The addition of sucrose or beta lactose resulted in cakes of larger volumes, and these cakes were more porous in structure. The volume of cakes containing 15% glucose or alpha lactose was slightly smaller, and the structure of these cakes was less coarse than those which had additions of the other two sugars. The cakes which contained 20% dried whey were slightly lower in volume than those of the basic formula. Previous work done in this laboratory (3) had shown that the best results were obtained when 10 to 15% whey was added to a cake formula. In the present study, it was felt necessary to add at least 15% of the various sugars in order to produce significant differences among the samples. The higher amount of whey was used, therefore, in order to have comparable sugar additions in all cakes.

Compressibility. The addition of 15% sucrose, glucose, alpha lactose, or beta lactose to the basic formula resulted in increased compressibility, but, as would be expected, these increases were not as great as when 30% of the sugars was added. As was the case in Section I, the cakes containing beta lactose were the softest at all times after baking. The addition of 20% dried whey also increased compressibility, and these cakes compared favorably with the cakes that had additions of the various sugars.

TABLE II

QUALITY CHARACTERISTICS OF CAKES OF SECTION II

	Basic Formula	Additions of 15% Various Sugars or 20% Dried Whey to the Basic Formula				
		Sucrose	Glucose	Alpha Lactose	Beta Lactose	Dried Whey
Batter viscosity Average	530	520	535	530	526	520
Volume (ml.) Average	314	330	325	324	330	311
Specific gravity Average	0.33	0.31	0.32	0.32	0.31	0.33
Compressibility Ratios						
3 hours	0.35	0.50	0.48	0.46	0.54	0.49
24 hours	0.28	0.33	0.37	0.36	0.41	0.40
72 hours	0.20	0.25	0.24	0.25	0.29	0.27
120 hours	0.17	0.20	0.21	0.22	0.26	0.22
Crust Color[1] Average	47.0	46.5	23.8	33.3	33.1	35.9
Average Scores 24 Hours after Baking						
Grain	16.1	15.2	15.6	16.8	15.4	17.1
Texture	15.5	15.2	16.5	16.9	15.7	17.5
Tenderness	12.4	11.9	12.8	12.7	12.5	13.3
Flavor	19.7	19.4	20.3	20.4	20.1	20.6
Sum of scores[2]	63.7	61.7	65.2	66.8	63.7	68.5
Average Scores 72 Hours after Baking						
Grain	16.6	14.4	14.9	14.8	15.3	16.1
Texture	14.9	14.5	15.2	14.5	14.5	16.1
Tenderness	12.3	12.2	12.8	12.5	12.6	13.2
Flavor	19.9	19.0	19.5	19.7	20.1	20.5
Sum of scores[2]	63.7	60.1	62.6	61.5	62.5	65.9

[1] Readings are given as % reflectance; magnesium oxide standard equals 100%. Thus, a lower value indicates a darker brown color.

[2] Maximum scores in all characteristics would give a total of 80.

Crust Color. It was observed in the first section of this study, that the addition of 30% of the various sugars resulted in striking variations in the crust colors of the cakes. Since this was also true when 15% of the sugars was added, an objective measurement of the differences in crust color was made using a Hunter Multipurpose Reflectometer. The results, summarized in Table II, reveal that the addition

of glucose to the basic formula resulted in cakes of the darkest crust color. The cakes which contained only sucrose were pale in color, and those which contained the two forms of lactose or dried whey were intermediate in color with crusts that were golden brown. These differences in crust color may be explained, in part, by the "browning reaction," as there seems to be a direct correlation between the relative number of free aldehyde groups present in the different sugars and the degree of color produced in the crusts.

TABLE III

ANALYSES OF VARIANCE FOR TEXTURE AND TENDERNESS SCORES OF CAKES OF SECTION II

Source of Variation	Degrees of Freedom	Mean Square	
		Texture Scores	Tenderness Scores
Among replications	5	17.46**	6.26**
Between times	1	143.09**	0.68
Among treatments	5	17.74**	7.18**
1) Among sugars	3)	2.70	2.28
2) Whey vs. mean of four sugars	1)	63.04**	24.38**
3) Sucrose vs. basic formula	1)	0.26	0.09
Replications x Times	5	7.44*	1.41*
Replications x Treatments	25	1.76	0.67
Times x Treatments	5	2.41	1.78*
Error (a)	25	2.20	0.53
Among judges	3	149.36**	16.32**
Judges X Replications	15	14.18**	1.66*
Judges X Times	3	3.94	1.38
Judges X Treatments	15	3.82*	1.12
Pooled error (b)	180	1.83	0.75
Total	287		

*Significant at 5% level of significance.
**Significant at 1% level of significance.

Scoring Panel. The judges' scores for cakes prepared in this section were analyzed statistically by analysis of variance for a split plot design. These data are presented in part in Table III.

There was a large variation among judges which was most pronounced for grain and least evident in the tenderness scores. The differences between the two storage times was significant for texture and tenderness, although not for flavor. The difference between storage times for grain scores was also significant. However, it is doubtful

that the variation here was actually due to hours of storage. It seems more reasonable to surmise that the differences between storage times, and also the large variation among judges for grain scores, were due to the fact that grain differed between cuts, or between cakes of the same baking and formula, none of which were tested in the experimental plan.

"Among Treatment" differences were statistically significant for all characteristics. The difference "Among Sugars" was statistically significant for tenderness, highly significant for grain and texture, but non-significant for flavor. The differences between the higher and lower amounts of sucrose were statistically significant only for grain. In the opinion of the judges, the higher sucrose cakes had less desirable grain.

Whey gave a highly significant difference from the mean of the sugars for all characteristics scored by the judges. Since the cakes containing whey generally received higher scores than any of the other samples, it may be concluded that the addition of dried whey to the basic formula resulted in a significant improvement in the quality of the cakes.

In this series, a comparison can be made of cakes made with the two forms of lactose and with the batter viscosity approximately equal. The volumes also were nearly equal but the cakes made with beta lactose were more compressible at every storage period. However, the scoring panel assigned lower scores especially for grain and texture of the beta lactose cakes 24 hours old but not at 72 hours. Again, it must be assumed that the variation in judges' reactions and the sampling errors account for the different scores for grain. Further work will have to indicate whether or not the higher scores for texture of the alpha lactose cakes at 24 hours was a real improvement or resulted in part from the additional water in the batter.

Additional indications of the judges' opinion of the different samples were obtained from the comments that were made on the score sheets during the judging periods. The cakes which contained dried whey received the greatest number of favorable comments, ten in all, and were considered to be moist and tender by many of the judges. This preference for the cakes which contained whey is verified by the higher numerical scores that were received by these cakes at both 24 and 72 hours after baking.

In contrast to the preference for "whey" cakes, the cakes which contained either level of sucrose received many comments which were, for the most part, unfavorable, and dryness seemed to be the outstanding characteristic of these cakes in comparison with the other samples. The cakes which had additions of 15% sucrose also received the

lowest scores for all characteristics. The other cakes, having additions of glucose, alpha lactose, or beta lactose, received only a few comments. Thus, from the comments and the numerical scores, it may be concluded that, in the opinion of the judging panel, the addition of 15% sucrose to the basic formula did not improve the overall quality of the cakes. The addition of glucose, alpha lactose, or beta lactose resulted in a small degree of improvement, but the greatest improvement resulted from the addition of 20% dried whey.

Acknowledgment

Thanks are due Dr. R. G. D. Steel, Agricultural Experiment Station Statistician for the statistical analyses.

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A NOTE ON THE SUGARS IN RICE[1]

KENNETH T. WILLIAMS AND ARTHUR BEVENUE

A study of quality changes in rice and its products during storage is being made by others in this Laboratory. This study requires some knowledge of the sugars present and their change during storage. Since the technical literature apparently contains no complete identification of the sugars in rice, and but little information on the total amount of sugar present in American-grown rice (1, 3), the authors have subjected a group of domestic rice samples to chemical and paper chromatographic analyses in order to obtain more information on the sugar components.

[1] Manuscript received December 15, 1952. Contribution from the Western Regional Research Laboratory, Albany, California, one of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U.S. Department of Agriculture.

Samples of brown, white, and parboiled rices of both the California Pearl and Texas Century Patna varieties, and samples of the brown and white rices of the waxy variety, California Mochi Gome, were used in the studies. All were from the 1952 crop. A single lot of rough rice of each variety was used in preparing the brown, white, and parboiled samples. The parboiled rices were commercial products. The brown and white rices were prepared in the laboratory by shelling and milling in accord with U.S. Department of Agriculture grading instructions (4), followed by air-cleaning and removal of all foreign material. All samples (12-15% moisture content) were passed through a 40-mesh sieve, and the sugars were extracted with 80% ethanol and quantitatively determined by methods previously described (6). The sugars were identified by paper chromatographic techniques (6).

The amounts of reducing and total sugars found in the rice samples are given in Table I. Hydrolysis by either invertase or hydrochloric acid gave equivalent total-sugar values.

TABLE I

SUGAR CONTENT OF RICE SAMPLES[1]

Variety and Type of Rice	Reducing[2] Sugars	Total Sugars[2]	
		Invertase Hydrolysis	HCl Hydrolysis
	%	%	%
California pearl			
Brown	0.13	1.08	1.10
White	0.07	0.50	0.51
Parboiled	0.14	1.02	1.06
Texas century patna			
Brown	0.09	0.83	0.83
White	0.05	0.37	0.37
Parboiled	0.19	0.65	0.66
California mochi gome			
Brown	0.11	1.36	1.40
White	0.08	0.53	0.53

[1] Analytical data on a moisture-free basis.
[2] As glucose.

Paper chromatography showed that all of the rice samples gave the same sugar pattern. The reducing-sugar portion was almost entirely glucose; the only other reducing sugar was a trace amount of fructose. All samples contained a trace amount of raffinose but the principal sugar was sucrose. Because raffinose exists in trace amounts only, the

difference between the reducing and total sugar values, given in Table I, may be considered to be the sucrose value. It was of interest to note that, unlike the wheat grain, no sugar larger than the trisaccharide, raffinose, was found in rice (2, 6).

The reported values for the sugars in brown, white, and parboiled rice are strictly comparable, as all the samples of each variety were from the same original source. However, because a single lot of each variety was used, no estimate is possible of the variability in a single variety due to cultural differences.

The data in Table I suggest that sugars from the bran layer of the brown rice migrate into the interior of the rice grain during the period of parboiling and remain in that part of the grain during subsequent drying and milling of the parboiled brown rice. Similar observations on the changes in the content of vitamins, minerals, and other water-soluble nutrients have been made by other workers (5, 7). However, there are some differences of opinion regarding the mechanisms involved in these changes (5).

Acknowledgment

The authors are indebted to Drs. E. B. Kester and D. F. Houston for helpful suggestions and for the samples of rice.

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COMMUNICATION TO THE EDITOR

Some Effects of Glutathione on the Tailings Starch Fraction of Wheat Flour and on Bread Made with This Fraction

DEAR SIR:

The action of glutathione in dough has been the subject of numerous investigations. While several theories have been proposed to explain the effect, most of the experimental evidence indicates that glutathione acts upon the protein of flour.

The possibility that the action of glutathione in baking is not entirely on the flour protein was suggested by experiments of Baker, Parker, and Mize,[1] who found that the effect of glutathione on purified soluble pentosans of flour was similar to its effect on dough. That observation suggested that glutathione might have a comparable effect on the tailings starch (or so-called amylodextrin) fraction of flour, which is relatively rich in cold-water dispersible but insoluble pentosans. Similarly the adverse effect of glutathione on bread quality might be due in part to its action on the pentosans of flour. The following experiments were undertaken to study the effect of glutathione on tailings starch and on bread made by use of this fraction.

Glutathione lowered the consistency appreciably, as shown in Table I, when tailings wheat starch was pasted in water buffered to pH 5.3 to 5.4. When large-granule wheat starch was pasted in a similar manner no effect due to glutathione was observed.

TABLE I

CONSISTENCY OF WHEAT STARCH FRACTIONS, BRABENDER AMYLOGRAPH UNITS

Wheat Starch Fraction	No glutathione		Glutathione, 0.1 g.	
	Maximum Reading	After 30 Minutes	Maximum Reading	After 30 Minutes
Tailings[1]	855	365	725	280
Large-granule[2]	810	730	810	740

[1] 80.0 g. tailings starch and 450 ml. buffered water. Maximum temperature 90°C. Average of two tests.
[2] 52.0 g. large-granule starch and 450 ml. buffered water. Maximum temperature 90°C. Average of five tests.

To study the effect of glutathione on the flour fractions in bread,

[1] Baker, J. C., Parker, H. K., and Mize, M. D. The pentosans of wheat flour. Cereal Chem. 20: 267-280 (1943).

flour was fractionated into gluten, large-granule and tailings wheat starch. The solubles were discarded. Breads were made using (a) the original flour, (b) gluten and large-granule wheat starch instead of flour, and (c) gluten, large-granule and tailings wheat starch instead of flour. The procedures of flour fractionation and baking of bread from the fractions are soon to appear in the literature.

As shown in Table II, glutathione lowered the specific volume of the bread made with flour and that made with either gluten and large-granule starch or gluten and a mixture of 4 parts large-granule to 1 part tailings starch. This agrees with the view that glutathione has an adverse effect upon the protein of flour. However, the effect of glutathione on specific volume was greatest in bread 4, which contained tailings starch.

TABLE II

EFFECT OF GLUTATHIONE ON THE VOLUME OF BREAD

Bread No.	Description of Bread	Specific Volume
		cu. in./oz.
1.	Made from gluten and large-granule wheat starch.	10.4
2.	Made from gluten, large-granule and tailings wheat starch.	10.3
3.	Same as No. 1, with 0.015% glutathione added.	9.2
4.	Same as No. 2, with 0.015% glutathione added.	8.1
5.	Made from flour.	10.3
6.	Made from flour, with 0.015% glutathione added.	8.6

These breads are shown in Fig. 1. The crumb of breads 1, 2, and 5, which did not contain glutathione, had fine grain and thin cell walls. Bread 3, which contained glutathione but no tailings starch, was likewise fine grained with thin cell walls. Bread 4, which contained both tailings starch and glutathione, and bread 6, made of flour with glutathione added, were very coarse grained with thick cell walls. Thus the effect of glutathione on the grain and texture of bread 4 was not an artifact caused by the process of fractionation of flour.

Other experiments, in which the same proportion of cystein was used, gave results similar to those with glutathione both with respect to the decrease in loaf volume and the adverse effect on crumb characteristics of bread which contained tailings starch.

It is not known which of the constituents of the tailings starch fraction was acted upon by glutathione to produce the great change in crumb characteristics observed in bread 4, and the decrease in consistency of the cooked starch. The observations suggest that it would

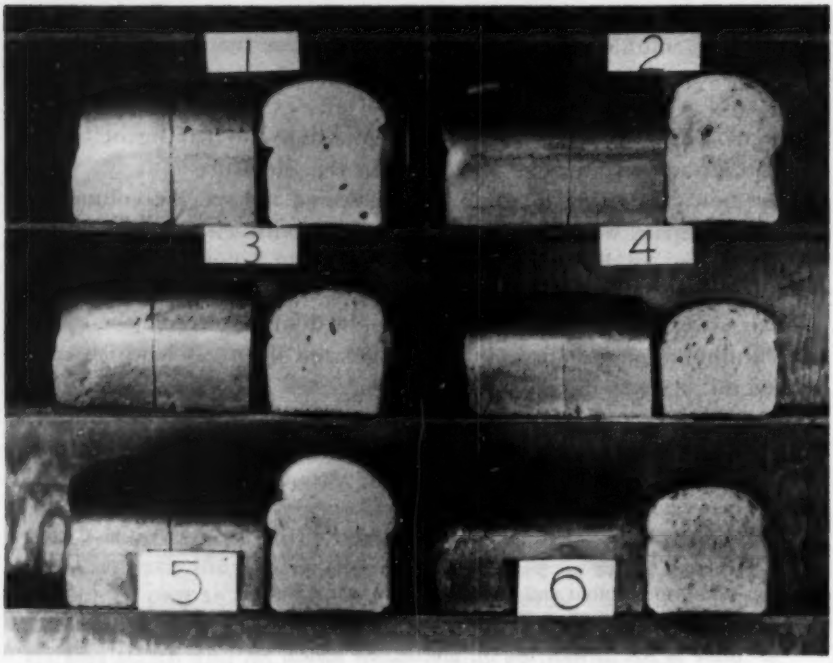

Fig. 1. Some effects of glutathione on the tailings fraction of wheat starch.

be of value to study separately the purified protein, starch, and pentosans derived from the tailings starch fraction.

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January 2, 1953

Advances in Agronomy. Vol. IV. Edited by A. G. Norman, 416 pp., Academic Press Inc., New York, New York, 1952. Price: $8.50.

This is the fourth in a series of contributions on important agronomic subjects. As in the past, the volume is edited by A. G. Norman with the help of an advisory board from the American Society of Agronomy, under the auspices of which the volume was prepared.

In this volume there are nine chapters. The first chapter is entitled "Grassland Agronomy in Australia" and is prepared by H. C. Trumbull, Waite Agricultural Research Institute, University of Adelaide. This contribution continues the pattern established in Volume III of including a review of agronomic developments and trends in particular countries or areas. Professor Trumbull is well qualified to prepare this particular review, both because of his long association with and leadership in grassland research in Australia and because of the world-wide experience he gained through service with the Food and Agriculture Organization.

The second review, "Type of Soil Colloid and the Mineral Nutrition of Plants," was prepared by A. Mehlich and N. T. Coleman of the North Carolina Agricultural Experiment Station. This paper is a well-prepared review of a vast amount of literature on the subject. In the introduction, consideration is given to classification of soil colloids, aspects of ion absorption from solutions, and ion antagonism or competition. A second section deals with approaches to the study of ionic environment of plant roots in soil, covering such subjects as ion exchange and ion activity. In the third section of the paper, the growth and cation contents of plants grown on natural and synthetic soils is considered. In this section, degree of base saturation, associated metal cations, cation exchange capacity, and ecological relationships of plants are considered. A fourth section of the paper deals with agronomic applications of the findings.

D. J. Watson, Rothamsted Experimental Station, prepared the third review, "The Physiological Basis of Variation in Yield." The author points out the problems involved in measurement of yield in experimentation and the importance of attempting to place yield variations on a physiological basis. Use and limitations of net assimilation rate (NAR), which is the rate of increase of dry weight per unit leaf area and is a measure of the excess of the rate of photosynthesis over the rate of dry matter loss by respiration, is discussed. Contrary to results of earlier workers, this author concludes that NAR varies, between and within species, with mineral nutrition and water supply and very widely with seasonal climatic conditions. Another unit of measurement discussed in the review is leaf area index which is an expression of the area of leaf surface per unit area of land surface. The author concludes that dry matter yields depend more on variation of leaf area than on NAR. Important problems yet to be solved are discussed in the latter part of the review.

"Copper and Nutrition" prepared by Frank A. Gilbert, Battelle Memorial Institute, is the fourth review. In addition to giving a historical sketch, this review deals with value of copper to plants, effects of copper deficiency in plants, copper in the soil, copper in animals, and regions of copper deficiency. Under copper in the soil, there are discussions of amount, factors influencing availability, effect on crop yields, comparisons of different forms of copper for use of soil amendment, residual effects, possible toxic effects, and effect on availability of other elements. Under the subject of copper in animals, use of copper in the body, deficiency symptoms, use of copper in mineral supplements, and toxicity, are discussed.

The fifth chapter, "Ecological and Physiological Factors in Compounding Forage Seed Mixtures," was prepared by Blaser, Skrdla, and Taylor of the Virginia Polytechnic Institute. In this review, the authors deal with problems related to artificial seed mixtures, plant adaptation as related to compounding seed mixtures, and use as a factor in compounding seed mixtures. Under plant adaptation, the authors consider climatic, soil, biological, and plant succession factors. The climatic factors are temperature and light, the soil factors moisture, aeration, soil fertility, and soil reaction, and the biological factors are plant morphology, palatability, diseases and insects, and crop varieties. Topics discussed under plant succession or the inter-relationship of climatic, soil, and biological factors, are grazing management of grass legume mixtures, improving degenerated Kentucky bluegrass-white clover pastures, competition for potassium, and seedling competi-

tion. In relation to use, the authors discuss yield and quality of forage as influenced by mixtures under the headings of "Top vs. Bottom Species" and of "Simple vs. Complex Mixtures." Of interest in the latter regard was the conclusion of the authors that no evidence has been submitted showing the superiority of complex over simple mixtures. The review is concluded by a section on developing a forage cropping system.

The sixth chapter, "Soil Manganese in Relation to Plant Growth," was prepared by E. G. Mulder and F. G. Gerretsen, Agricultural Experiment Station and Institute of Soil Research, Groningen, The Netherlands. In this paper the authors have reviewed methods of manganese determination, the occurrence and availability of manganese in soil and factors affecting its availability, the role of microorganisms in transforming manganese compounds, symptoms of manganese deficiency in plants, manganese content of plants, correcting manganese deficiency, manganese nutrition and fertilizer interactions, manganese toxicity in plants, and function of manganese in plants. This would appear to be a comprehensive treatment of the subject.

N. Edward Tobert and Paul B. Pearson of the United States Atomic Energy Commission have prepared the seventh paper entitled "Atomic Energy and the Plant Sciences." This is a review of the work on this subject supported by the Atomic Energy Commission in national laboratories and through grants in universities and colleges. As a result the review cannot be looked upon as a comprehensive treatment of the subject of applications of atomic energy in the plant sciences. The review treats the effects of radiation on plants including external radiation and plant development, internal radiation effects, genetic effects in plant breeding, biochemical effects of radiation, and biological chains. It also treats the use of isotopes in plant sciences including soil, fertilizer, and mineral nutrition, and plant metabolism.

The eighth chapter, "Vegetation Control on Industrial Land," was prepared by Keith C. Barrons of the Dow Chemical Company. The chapter is devoted largely to advances in use of herbicides for the control of unwanted vegetation. In the first part of the chapter, the various herbicides that have already found a place are discussed. The most extensive discussion is of the chlorophenoxyacetic acids of which the two best known are 2, 4-D and 2,4,5-T. For this group, derivatives and formulations, some physiologic aspects, specificity of the compounds, and methods of application are discussed. Other herbicides discussed are sodium chlorate, sodium trichloroacetate, substituted phenols, herbicidal oils, boron compound, sodium arsenite, ammonium sulfamate, CMU, and mixtures of herbicides. The review concludes with a discussion of some of the special problems involved in vegation control on various types of industrial lands including railroad, highway, pipeline, electric line, telephone line, and telegraph line right of ways, transmitter and transformer stations, drainage and irrigation ditches, grounds surrounding various industrial establishments, airfields, etc.

The ninth chapter, "Soil and the Growth of the Forest," was prepared by T. S. Coile, Duke University. In the introduction to this chapter, the author deals with a general statement of the problem, measures of forest growth, and soil properties related to forest growth. In the second section, entitled "Soil as an Environment for Tree Roots," the author discusses effectiveness and distribution of precipitation, soil properties, topography, and the inherent rooting habit of forest trees. The site index and other features of forest growth are discussed in relation to the soil for various regions including the Northwestern region, Lake States, Central hardwood, Prairie-plains, Southern Appalachian, Southern, and Southeastern regions. The review is concluded with a resumé of principal soil and other factors related to forest growth.

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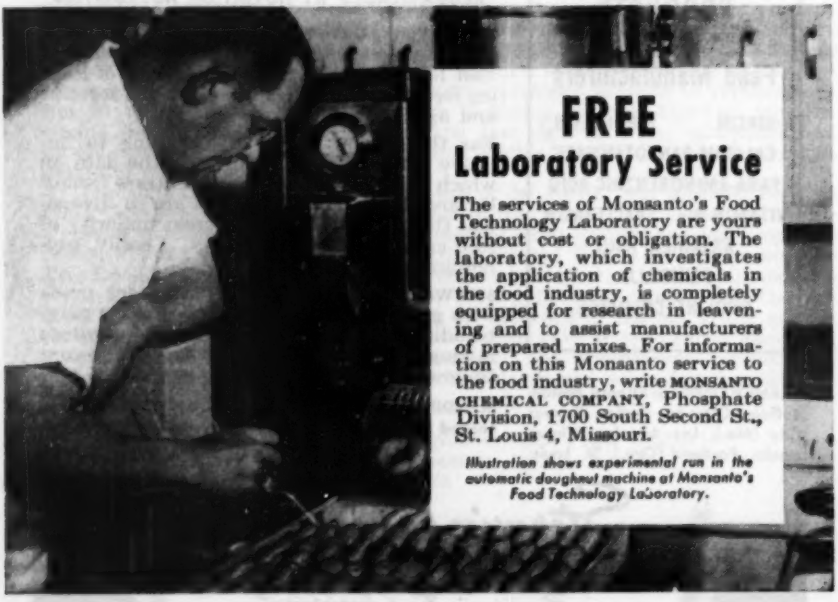

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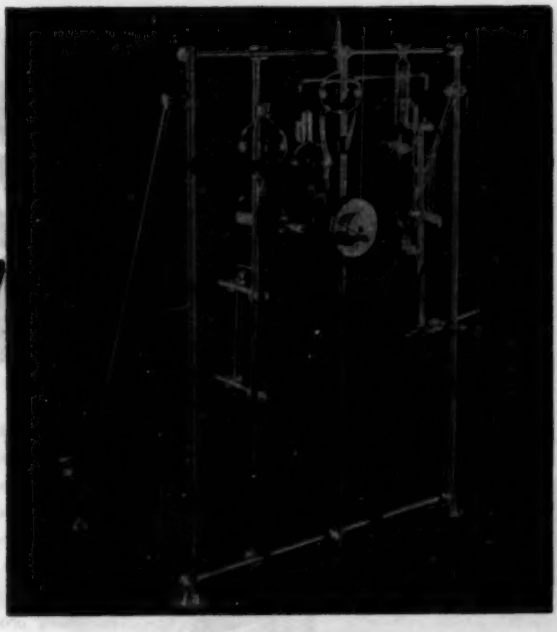

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